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(54) Title: PHARMACEUTICAL COMPOSITIONS OF DRUG-OLIGOMER CONJUGATES AND METHODS OF TREATING DISEASES THEREWITH

(57) Abstract: Pharmaceutical compositions that include a drug-oligomer conjugate, a fatty acid component, and a bile salt component are described. The drug is covalently coupled to an oligomeric moiety. The fatty acid component and the bile salt component are present in a weight-to-weight ratio of between 1:5 and 5:1. Methods of treating diseases in a subject in need of such treatment using such pharmaceutical compositions are also provided, as are methods of providing such pharmaceutical compositions.

PHARMACEUTICAL COMPOSITIONS OF DRUG-OLIGOMER CONJUGATES AND METHODS OF TREATING DISEASES THEREWITH

Related Application

This application claims the benefit of United States Provisional Application Number 60/318,193, filed September 7, 2001 and United States Provisional Application Number 60/377,865, filed May 3, 2002, the disclosures of which are incorporated herein by reference in their entireties.

Field of the Invention

The present invention relates to pharmaceutical compositions and methods of treating diseases therewith.

Background of the Invention

Fatty acids have been proposed as excipients in pharmaceutical compositions of unconjugated drug molecules. For example, U.S. Patent No. 4,338,306 to Kitao et al. proposes pharmaceutical compositions for rectal administration of insulin. The pharmaceutical compositions include insulin and fatty acids having 8 to 14 carbon atoms and nontoxic salts thereof.

U.S. Patent No. 5,658,878 to Bäckström et al. proposes a therapeutic preparation for inhalation that includes insulin and a substance which enhances the absorption of insulin in the lower respiratory tract. The enhancer is preferably a sodium salt of a saturated fatty acid of carbon chain length 10 (i.e., sodium caprate), 12 (sodium laurate), or 14 (sodium myristate). Potassium and lysine salts of capric acid are also proposed. Bäckström et al. note that if the carbon chain length is shorter than about 10, the surface activity of the surfactant may be too low, and if the chain length is longer than about 14, decreased solubility of the fatty acid in water limits its usefulness. As an alternative to the proposed fatty acid

enhancers, Bäckström et al. propose the use of the following bile salts - sodium ursodeoxycholate, sodium taurocholate, sodium glycocholate, and sodium taurodihydrofusidate.

U.S. Patent No. 6,200,602 to Watts et al. proposes drug delivery compositions for colonic delivery of insulin. The drug delivery compositions include insulin, an absorption promoter which (a) includes a mixture of fatty acids having 6 to 16 carbon atoms or a salt thereof and a dispersing agent, or (b) comprises a mixture of mono/diglycerides of medium chain fatty acids and a dispersing agent, and a coating to prevent the release of the insulin and absorption promoter until the tablet, capsule or pellet reaches the proximal colon.

Bile salts have been proposed as excipients in pharmaceutical compositions of unconjugated drug molecules. For example, U.S. Patent No. 4,579,730 to Kidron et al. proposes pharmaceutical compositions for the oral administration of insulin. The pharmaceutical compositions include insulin, a bile acid or alkali metal salt thereof, the bile acid being selected from the group consisting of cholic acid, chenodeoxycholic acid, taurocholic acid, taurochenodeoxycholic acid, glycocholic acid, glycochenocholic acid, 3β-hydroxy-12-ketocholic acid, 12α-3β-dihydrocholic acid, and ursodesoxycholic acid, and a protease inhibitor. The composition is provided with an enterocoating to assure passage through the stomach and release in the intestine.

U.S. Patent No. 5,283,236 to Chiou proposes compositions for systemic delivery of insulin through the eyes where the drug passes into the nasolacrimal duct and becomes absorbed into circulation. The composition includes insulin and an enhancing agent. The enhancing agents proposed include, either alone or in combination, surfactants such as polyoxyethylene ethers of fatty acids, and bile salts and acids such as cholic acid, deoxycholic acid, glycocholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, sodium cholate, sodium glycocholate, glycocholate, sodium deoxycholate, sodium taurodeoxycholate, chenodeoxycholic acid, and ursodeoxycholic acid. The enhancer is present in a concentration ranging from 0.1% to 5% (w/v).

U.S. Patent No. 5,853,748 to New proposes enteric-coated compositions for oral administration of insulin. The composition includes insulin, a bile salt or bile acid, and carbonate or bicarbonate ions, which are used to adjust the pH of the gut to a pH of from 7.5 to 9.

In at least one instance, a mixture of a low detergent bile salt, such as ursodeoxycholate, and a C_{12} to C_{24} fatty acid, such as linoleic or oleic acid, has been proposed as excipients in a pharmaceutical composition of orally acitve pharmaceutically

active agents that need to be protected from the acidic and enzymatic environment of the stomach and for which the gastrointestinal mucosa should be protected from adverse effects of the drug. The proposed pharmaceutical composition is proposed to be particularly suitable for the non-steroidal anti-inflammatory drug indomethacin.

It is desirable to provide pharmaceutical compositions for administration of drug-oligomer conjugates.

Summary of the Invention

Pharmaceutical compositions according to embodiments of the present invention use a mixture of bile salts and fatty acids in a particular ratio that appears to provide synergistic effects in the administration of drug-oligomer conjugates that may not be achieved with bile salts or fatty acids alone. For example, in some embodiments of the present invention, using mixtures of bile salts and fatty acids in a particular ratio alters the precipitation characteristics of the bile salt so that the bile salt more readily re-solubilizes if it happens to precipitate out of the pharmaceutical composition (e.g., upon encountering an acidic environment in the gut). As another example, in some embodiments of the present invention, using mixtures of bile salts and fatty acids in a particular ratio lowers the precipitation point of the bile salt in the pharmaceutical composition, providing additional buffering capacity for the pharmaceutical composition.

According to embodiments of the present invention, a pharmaceutical composition includes a drug-oligomer conjugate that includes a drug covalently coupled to an oligomeric moiety, a fatty acid component that includes a fatty acid, and a bile salt component that includes a bile salt. The fatty acid component and the bile salt component are present in a weight-to-weight ratio of between 1:5 and 5:1. The fatty acid component is present in an amount sufficient to lower the precipitation point of the bile salt compared to a precipitation point of the bile salt if the fatty acid component were not present in the pharmaceutical composition. The bile salt component is present in an amount sufficient to lower the solubility point of the fatty acid compared to a solubility point of the fatty acid if the bile salt were not present in the pharmaceutical composition.

According to other embodiments of the present invention, a pharmaceutical composition includes a drug-oligomer conjugate that includes a drug covalently coupled to an oligomeric moiety, a bile salt component that includes a bile salt, and a fatty acid component that includes a fatty acid. The fatty acid component and the bile salt component are present in a weight-to-weight ratio of between 1:5 and 5:1. The fatty acid component is present in a

first amount such that, at the precipitation point of the bile salt, the bile salt precipitates as first bile salt particles that, upon a return to a pH above the precipitation point of the bile salt, re-solubilize more quickly than second bile salt particles that would have precipitated if the fatty acid component were present in a second amount less than the first amount.

According to still other embodiments of the present invention, a pharmaceutical composition includes a drug-oligomer conjugate that includes a drug covalently coupled to an oligomeric moiety, between 0.1 and 15% (w/v) of a fatty acid component, and between 0.1 and 15% (w/v) of a bile salt component. The fatty acid component and the bile salt component are present in a weight-to-weight ratio of between 1:5 and 5:1.

According to other embodiments of the present invention, methods of treating a disease state in a subject in need of such treatment include administering to the subject a pharmaceutical composition according to embodiments of the present invention.

According to still other embodiments of the present invention, a method of providing a pharmaceutical composition includes selecting an amount of a bile salt to include in the composition based on the ability of the bile salt to increase the solubility of a fatty acid component when the composition has a pH of 8.5 or less.

According to yet other embodiments of the present invention, a method of providing a pharmaceutical composition includes selecting an amount of a fatty acid to include in the composition based on the ability of the fatty acid to lower the precipitation point of a bile salt component in the composition to a pH of 5.5 or less.

According to other embodiments of the present invention, a method of providing a pharmaceutical composition includes selecting an amount of a fatty acid to include in the composition based on the ability of the fatty acid to alter the precipitation characteristics of a bile salt component in the composition.

Brief Description of the Drawings

Figure 1 illustrates a comparison of mean plasma glucose vs. time curves resulting from oral administration of various doses of embodiments of the present invention in fasting, non-diabetic subjects compared with a mean plasma glucose vs. time curve for baseline plasma glucose;

Figure 2 illustrates a comparison of mean plasma insulin vs. time curve resulting from oral administration of various doses of embodiments of the present invention in fasting, non-diabetic subjects compared with a mean plasma insulin vs. time curve for baseline plasma insulin;

Figure 3 illustrates glucose and insulin dose responses resulting from oral administration of embodiments of the present invention in fasting, non-diabetic subjects;

Figure 4 illustrates a comparison of mean plasma glucose vs. time curves resulting from post-prandial, oral administration of various doses of embodiments of the present invention in non-diabetic subjects compared with a mean plasma glucose vs. time curve for baseline plasma glucose;

Figure 5 illustrates a comparison of mean plasma insulin vs. time curve resulting from post-prandial, oral administration of various doses of embodiments of the present invention in non-diabetic subjects compared with a mean plasma insulin vs. time curve for baseline plasma insulin;

Figure 6 illustrates glucose and insulin dose responses resulting from post -prandial oral administration of embodiments of the present invention in non-diabetic subjects;

Figure 7 illustrates a blood glucose vs. time curve resulting from oral administration of embodiments of the present invention in mice;

Figure 8 illustrates a blood glucose vs. time curve resulting from oral administration of embodiments of the present invention in mice;

Figure 9 illustrates a blood glucose vs. time curve resulting from oral administration of embodiments of the present invention in mice;

Figure 10 illustrates a calcitonin serum level vs. time curve resulting from oral administration of liquid formulation embodiments of the present invention in beagle dogs;

Figure 11 illustrates a calcitonin serum level vs. time curve resulting from oral administration of liquid formulation embodiments of the present invention in beagle dogs;

Figure 12 illustrates a calcitonin serum level vs. time curve resulting from oral administration of solid formulation embodiments of the present invention in beagle dogs; and

Figure 13 illustrates a plasma concentration of C-peptide resulting from oral administration of liquid formulation embodiments of the present invention in mice.

Detailed Description of Preferred Embodiments

The present invention now will be described more fully hereinafter with reference to the accompanying drawings, in which preferred embodiments of the invention are shown. This invention may, however, be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art.

All amino acid abbreviations used in this disclosure are those accepted by the United States Patent and Trademark Office as set forth in 37 C.F.R. § 1.822(b).

As used herein, the term "between" when used to describe various ranges should be interpreted to include the end-points of the described ranges.

As used herein, the term "substantially monodispersed" is used to describe a mixture of compounds wherein at least about 95 percent of the compounds in the mixture have the same molecular weight.

As used herein, the term "monodispersed" is used to describe a mixture of compounds wherein about 100 percent of the compounds in the mixture have the same molecular weight.

As used herein, the term "drug" refers to any therapeutic compound that is conjugatable in the manner of the present invention. Representative non-limiting classes of therapeutic compounds useful in the present invention include those falling into the following therapeutic categories: ACE-inhibitors; anti-anginal drugs; anti-arrhythmias; anti-asthmatics; anti-cancer drugs; anti-cholesterolemics; anti-convulsants; anti-depressants; anti-diarrhea preparations; anti-histamines; anti-hypertensive drugs; anti-infectives; anti-inflammatory agents; anti-lipid agents; anti-manics; anti-nauseants; anti-stroke agents; anti-thyroid preparations; anti-tumor drugs; anti-tussives; anti-uricemic drugs; anti-viral agents; acne drugs; alkaloids; amino acid preparations; anabolic drugs; analgesics; anesthetics; angiogenesis inhibitors; antacids; anti-arthritics; antibiotics; anticoagulants; antiemetics; antiobesity drugs; antiparasitics; antipsychotics; antipyretics; antispasmodics; antithrombotic drugs; anxiolytic agents; appetite stimulants; appetite suppressants; beta blocking agents; bronchodilators; cardiovascular agents; cerebral dilators; chelating agents; cholecystokinin antagonists; chemotherapeutic agents; cognition activators; contraceptives; coronary dilators; cough suppressants; decongestants; deodorants; dermatological agents; diabetes agents; diuretics; emollients; enzymes; erythropoietic drugs; expectorants; fertility agents; fungicides; gastrointestinal agents; growth regulators; hormone replacement agents; hyperglycemic agents; hypnotics; hypoglycemic agents; laxatives; migraine treatments; mineral supplements; mucolytics; narcotics; neuroleptics; neuromuscular drugs; NSAIDS; nutritional additives; peripheral vasodilators; polypeptides; prostaglandins; psychotropics; renin inhibitors; respiratory stimulants; steroids; stimulants; sympatholytics; thyroid preparations; tranquilizers; uterine relaxants; vaginal preparations; vasoconstrictors; vasodilators; vertigo agents; vitamins; and wound healing agents.

The drug is preferably a polypeptide. Non-limiting examples of polypeptides that may be useful in the present invention include the following:

Adrenocorticotropic hormone (ACTH) peptides including, but not limited to, ACTH, human; ACTH 1-10; ACTH 1-13, human; ACTH 1-16, human; ACTH 1-17; ACTH 1-24, human; ACTH 4-10; ACTH 4-11; ACTH 6-24; ACTH 7-38, human; ACTH 18-39, human; ACTH, rat; ACTH 12-39, rat; beta-cell tropin (ACTH 22-39); biotinyl-ACTH 1-24, human; biotinyl-ACTH 7-38, human; corticostatin, human; corticostatin, rabbit; [Met(02)^4, DLys^8, Phe^9] ACTH 4-9, human; [Met(0)^4,DLys^8, Phe^9] ACTH 4-9, human; N-acetyl, ACTH 1-17, human; and ebiratide.

Adrenomedullin peptides including, but not limited to, adrenomedullin, adrenomedullin 1-52, human; adrenomedullin 1-12, human; adrenomedullin 13-52, human; adrenomedullin 22-52, human; pro-adrenomedullin 45-92, human; pro-adrenomedullin 153-185, human; adrenomedullin 1-52, porcine; pro-adrenomedullin (N-20), porcine; adrenomedullin 1-50, rat; adrenomedullin 11-50, rat; and proAM-N20 (proadrenomedullin N-terminal 20 peptide), rat.

Allatostatin peptides including, but not limited to, allatostatin I; allatostatin II; allatostatin III; and allatostatin IV.

Amylin peptides including, but not limited to, acetyl-amylin 8-37, human; acetylated amylin 8-37, rat; AC187 amylin antagonist; AC253 amylin antagonist; AC625 amylin antagonist; amylin 8-37, human; amylin (IAPP), cat; amylin (insulinoma or islet amyloid polypeptide(IAPP)); amylin amide, human; amylin 1-13 (diabetes-associated peptide 1-13), human; amylin 20-29 (IAPP 20-29), human; AC625 amylin antagonist; amylin 8-37, human; amylin (IAPP), cat; amylin, rat; amylin 8-37, rat; biotinyl-amylin, rat; and biotinyl-amylin amide, human.

Amyloid beta-protein fragment peptides including, but not limited to, Alzheimer's disease beta-protein 12-28 (SP17); amyloid beta-protein 25-35; amyloid beta/A4-protein precursor 328-332; amyloid beta/A4 protein precursor (APP) 319-335; amyloid beta-protein 1-43; amyloid beta-protein 1-42; amyloid beta-protein 1-40; amyloid beta-protein 10-20; amyloid beta-protein 22-35; Alzheimer's disease beta-protein (SP28); beta-amyloid peptide 1-42, rat; beta-amyloid peptide 1-40, rat; beta-amyloid 1-11; beta-amyloid 31-35; beta-amyloid 32-35; beta-amyloid 35-25; beta-amyloid/A4 protein precursor 96-110; beta-amyloid precursor protein 657-676; beta-amyloid 1-38; [Gln^{11}]-Alzheimer's disease beta-protein; [Gln^{11}]-beta-amyloid 1-40; [Gln^{22}]-beta-amyloid 6-40; non-A beta component of Alzheimer's disease amyloid (NAC); P3, (A beta 17-40) Alzheimer's disease amyloid β-peptide; and SAP (serum amyloid P component) 194-204.

Angiotensin peptides including, but not limited to, A-779; Ala-Pro-Gly-angiotensin

II; [Ile^3,Val^5]-angiotensin II; angiotensin III antipeptide; angiogenin fragment 108-122; angiogenin fragment 108-123; angiotensin I converting enzyme inhibitor; angiotensin I, human; angiotensin I converting enzyme substrate; angiotensin 11-7, human; angiopeptin; angiotensin II, human; angiotensin II antipeptide; angiotensin II 1-4, human; angiotensin II 3-8, human; angiotensin II 4-8, human; angiotensin II 5-8, human; angiotensin III ([Des-Asp^1]-angiotensin II), human; angiotensin III inhibitor ([Ile^7]-angiotensin III); angiotensin-converting enzyme inhibitor (Neothunnus macropterus); [Asn^1, Val^5]-angiotensin I, goosefish; [Asn^1, Val^5, Asn^9]-angiotensin I, salmon; [Asn^1, Val^5, Gly^9]-angiotensin I, eel; [Asn^1, Val^5]-angiotensin I 1-7, eel, goosefish, salmon; [Asn^1, Val^5]-angiotensin II; biotinyl-angiotensin I, human; biotinyl-angiotensin II, human; biotinyl-Ala-Ala-Ala-angiotensin II; [Des-Asp^1]-angiotensin I, human; [p-aminophenylalanine^6]-angiotensin II; renin substrate (angiotensinogen 1-13), human; preangiotensinogen 1-14 (renin substrate tetradecapeptide), human; renin substrate tetradecapeptide (angiotensinogen 1-14), porcine; [Sar^1]-angiotensin II, [Sar^1]-angiotensin II 1-7 amide; [Sar^1, Ala^8]-angiotensin II; [Sar^1, Ile^8]-angiotensin II; [Sar^1, Thr^8]-angiotensin II; [Sar^1, Tyr(Me)^4]-angiotensin II (Sarmesin); [Sar^1, Val^5, Ala^8]-angiotensin II; [Sar^1, Ile^7]-angiotensin III; synthetic tetradecapeptide renin substrate (No. 2); [Val^4]-angiotensin III; [Val^5]-angiotensin II; [Val^5]-angiotensin I, human; [Val^5]-angiotensin I; [Val^5, Asn^9]-angiotensin I, bullfrog; and [Val^5, Ser^9]-angiotensin I, fowl.

Antibiotic peptides including, but not limited to, Ac-SQNY; bactenecin, bovine; CAP 37 (20-44); carbormethoxycarbonyl-DPro-DPhe-OBzl; CD36 peptide P 139-155; CD36 peptide P 93-110; cecropin A-melittin hybrid peptide [CA(1-7)M(2-9)NH2]; cecropin B, free acid; CYS(Bzl)84 CD fragment 81-92; defensin (human) HNP-2; dermaseptin; immunostimulating peptide, human; lactoferricin, bovine (BLFC); and magainin spacer.

Antigenic polypeptides, which can elicit an enhanced immune response, enhance an immune response and or cause an immunizingly effective response to diseases and/or disease causing agents including, but not limited to, adenoviruses; anthrax; Bordetella pertussus; botulism; bovine rhinotracheitis; Branhamella catarrhalis; canine hepatitis; canine distemper; Chlamydiae; cholera; coccidiomycosis; cowpox; cytomegalovirus; Dengue fever; dengue toxoplasmosis; diphtheria; encephalitis; enterotoxigenic E. coli; Epstein Barr virus; equine encephalitis; equine infectious anemia; equine influenza; equine pneumonia; equine rhinovirus; Escherichia coli; feline leukemia; flavivirus; globulin; haemophilus influenza type b; Haemophilus influenzae; Haemophilus pertussis; Helicobacter pylon; hemophilus; hepatitis; hepatitis A; hepatitis B; Hepatitis C; herpes viruses; HIV; HIV- 1 viruses; HIV-2 viruses; HTLV; influenza; Japanese encephalitis; Klebsiellae species; Legionella

pneumophila; leishmania; leprosy; lyme disease; malaria immunogen; measles; meningitis; meningococcal; Meningococcal polysaccharide group A; Meningococcal polysaccharide group C; mumps; mumps virus; mycobacteria; Mycobacterium tuberculosis; Neisseria; Neisseria gonorrhea; Neisseria meningitidis; ovine blue tongue; ovine encephalitis; papilloma; parainfluenza; paramyxoviruses; Pertussis; plague; pneumococcus; Pneumocystis carinii; pneumonia; poliovirus; proteus species; Pseudomonas aeruginosa; rabies; respiratory syncytial virus; rotavirus; rubella; salmonellae; schistosomiasis; shigellae; simian immunodeficiency virus; smallpox; Staphylococcus aureus; Staphylococcus species; Streptococcus pneumoniae; Streptococcus pyogenes; Streptococcus species; swine influenza; tetanus; Treponema pallidum; typhoid; vaccinia; varicella-zoster virus; and vibrio cholerae.

Anti-microbial peptides including, but not limited to, buforin I; buforin II; cecropin A; cecropin B; cecropin P1, porcine; gaegurin 2 (Rana rugosa); gaegurin 5 (Rana rugosa); indolicidin; protegrin-(PG)-I; magainin 1; and magainin 2; and T-22 [$Tyr^{5,12}$, Lys^{7}]-poly-phemusin II peptide.

Apoptosis related peptides including, but not limited to, Alzheimer's disease beta-protein (SP28); calpain inhibitor peptide; capsase-1 inhibitor V; capsase-3, substrate IV; caspase-1 inhibitor I, cell-permeable; caspase-1 inhibitor VI; caspase-3 substrate III, fluorogenic; caspase-1 substrate V, fluorogenic; caspase-3 inhibitor I, cell-permeable; caspase-6 ICE inhibitor III; [Des-Ac, biotin]-ICE inhibitor III; IL-1 B converting enzyme (ICE) inhibitor II; IL-1 B converting enzyme (ICE) substrate IV; MDL 28170; and MG-132.

Atrial natriuretic peptides including, but not limited to, alpha-ANP (alpha-chANP), chicken; anantin; ANP 1-11, rat; ANP 8-30, frog; ANP 11-30, frog; ANP-21 (fANP-21), frog; ANP-24 (fANP-24), frog; ANP-30, frog; ANP fragment 5-28, human, canine; ANP-7-23, human; ANP fragment 7-28, human, canine; alpha-atrial natriuretic polypeptide 1-28, human, canine; A71915, rat; atrial natriuretic factor 8-33, rat; atrial natriuretic polypeptide 3-28, human; atrial natriuretic polypeptide 4-28, human, canine; atrial natriuretic polypeptide 5-27; human; atrial natriuretic aeptide (ANP), eel; atriopeptin I, rat, rabbit, mouse; atriopeptin II, rat, rabbit, mouse; atriopeptin III, rat, rabbit, mouse; atrial natriuretic factor (rANF), rat, auriculin A (rat ANF 126-149); auriculin B (rat ANF 126-150); beta-ANP (1-28, dimer, antiparallel); beta-rANF 17-48; biotinyl-alpha-ANP 1-28, human, canine; biotinyl-atrial natriuretic factor (biotinyl-rANF), rat; cardiodilatin 1-16, human; C-ANF 4-23, rat; Des-[Cys^{105}, Cys^{121}]-atrial natriuretic factor 104-126, rat; [$Met(O)^{12}$] ANP 1-28, human; [Mpr^{7},$DAla^{9}$]ANP 7-28, amide, rat; prepro-ANF 104-116, human; prepro-ANF 26-55 (proANF 1-30), human; prepro-ANF 56-92 (proANF 31-67), human; prepro-ANF 104-123,

human; [Tyr^0]-atriopeptin I, rat, rabbit, mouse; [Tyr^0]-atriopeptin II, rat, rabbit, mouse; [Tyr^0]-prepro ANF 104-123, human; urodilatin (CDD/ANP 95-126); ventricular natriuretic peptide (VNP), eel; and ventricular natriuretic peptide (VNP), rainbow trout.

Bag cell peptides including, but not limited to, alpha bag cell peptide; alpha-bag cell peptide 1-9; alpha-bag cell peptide 1-8; alpha-bag cell peptide 1-7; beta-bag cell factor; and gamma-bag cell factor.

Bombesin peptides including, but not limited to, alpha-s1 casein 101-123 (bovine milk); biotinyl-bombesin; bombesin 8-14; bombesin; [Leu^{13}-psi (CH2NH)Leu^{14}]-bombesin; [D-Phe^6, Des-Met^{14}]-bombesin 6-14 ethylamide; [DPhe^{12}] bombesin; [DPhe^{12},Leu^{14}]-bombesin; [Tyr^4]-bombesin; and [Tyr^4,DPhe^{12}]-bombesin.

Bone GLA peptides (BGP) including, but not limited to, bone GLA protein; bone GLA protein 45-49; [Glu^{17}, Gla^{21,24}]-osteocalcin 1-49, human; myclopeptide -2 (MP-2); osteocalcin 1-49 human; osteocalcin 37-49, human; and [Tyr^{38}, Phe^{42,46}] bone GLA protein 38-49, human.

Bradykinin peptides including, but not limited to, [Ala^{2,6}, des-Pro^3]-bradykinin; bradykinin; bradykinin (Bowfin. Gar); bradykinin potentiating peptide; bradykinin 1-3; bradykinin 1-5; bradykinin 1-6; bradykinin 1-7; bradykinin 2-7; bradykinin 2-9; [DPhe^7] bradykinin; [Des-Arg^9]-bradykinin; [Des-Arg^{10}]-Lys-bradykinin ([Des-Arg^{10}]-kallidin); [D-N-Me-Phe^7]-bradykinin; [Des-Arg^9, Leu^8]-bradykinin; Lys-bradykinin (kallidin); Lys-[Des-Arg^9,Leu^8]-bradykinin ([Des-Arg^{10},Leu^9]-kallidin); [Lys^0-Hyp^3]-bradykinin; ovokinin; [Lys^0, Ala^3]-bradykinin; Met-Lys-bradykinin; peptide K12 bradykinin potentiating peptide; [(pCl)Phe^{5,8}]-bradykinin; T-kinin (Ile-Ser-bradykinin); [Thi^{5,8}, D-Phe^7]-bradykinin; [Tyr^0]-bradykinin; [Tyr^5]-bradykinin; [Tyr^8]-bradykinin; and kallikrein.

Brain natriuretic peptides (BNP) including, but not limited to, BNP 32, canine; BNP-like Peptide, eel; BNP-32, human; BNP-45, mouse; BNP-26, porcine; BNP-32, porcine; biotinyl-BNP-32, porcine; BNP-32, rat; biotinyl-BNP-32, rat; BNP-45 (BNP 51-95, 5K cardiac natriuretic peptide), rat; and [Tyr^0]-BNP 1-32, human.

C-peptides including, but not limited to, C-peptide; and [Tyr^0]-C-peptide, human.

C-type natriuretic peptides (CNP) including, but not limited to, C-type natriuretic peptide, chicken; C-type natriuretic peptide-22 (CNP-22), porcine, rat, human; C-type natriuretic peptide-53 (CNP-53), human; C-type natriuretic peptide-53 (CNP-53), porcine, rat; C-type natriuretic peptide-53 (porcine, rat) 1-29 (CNP-53 1-29); prepro-CNP 1-27, rat; prepro-CNP 30-50, porcine, rat; vasonatrin peptide (VNP); and [Tyr^0]-C-type natriuretic peptide-22 ([Tyr^0]-CNP-22).

Calcitonin peptides including, but not limited to, biotinyl-calcitonin, human; biotinyl-calcitonin, rat; biotinyl-calcitonin, salmon; calcitonin, chicken; calcitonin, eel; calcitonin, human; calcitonin, porcine; calcitonin, rat; calcitonin, salmon; calcitonin 1-7, human; calcitonin 8-32, salmon; katacalcin (PDN-21) (C-procalcitonin); and N-proCT (amino-terminal procalcitonin cleavage peptide), human.

Calcitonin gene related peptides (CGRP) including, but not limited to, acetyl-alpha-CGRP 19-37, human; alpha-CGRP 19-37, human; alpha-CGRP 23-37, human; biotinyl-CGRP, human; biotinyl-CGRP II, human; biotinyl-CGRP, rat; beta-CGRP, rat; biotinyl-beta-CGRP, rat; CGRP, rat; CGRP, human; calcitonin C-terminal adjacent peptide; CGRP 1-19, human; CGRP 20-37, human; CGRP 8-37, human; CGRP II, human; CGRP, rat; CGRP 8-37, rat; CGRP 29-37, rat; CGRP 30-37, rat; CGRP 31-37, rat; CGRP 32-37, rat; CGRP 33-37, rat; CGRP 31-37, rat; ([Cys(Acm)^{2,7}]-CGRP; elcatonin; [Tyr^{0}]-CGRP, human; [Tyr^{0}]-CGRP II, human; [Tyr^{0}]-CGRP 28-37, rat; [Tyr^{0}]-CGRP, rat; and [Tyr^{22}]-CGRP 22-37, rat.

CART peptides including, but not limited to, CART, human; CART 55-102, human; CART, rat; and CART 55-102, rat.

Casomorphin peptides including, but not limited to, beta-casomorphin, human; beta-casomorphin 1-3; beta-casomorphin 1-3, amide; beta-casomorphin, bovine; beta-casomorphin 1-4, bovine; beta-casomorphin 1-5, bovine; beta-casomorphin 1-5, amide, bovine; beta-casomorphin 1-6, bovine; [DAla^{2}]-beta-casomorphin 1-3, amide, bovine; [DAla^{2},Hyp^{4},Tyr^{5}]-beta-casomorphin 1-5 amide; [DAla^{2},DPro^{4},Tyr^{5}]-beta-casomorphin 1-5, amide; [DAla^{2},Tyr^{5}]-beta-casomorphin 1-5, amide, bovine; [DAla^{2,4},Tyr^{5}]-beta-casomorphin 1-5, amide, bovine; [DAla^{2}, (pCl)Phe^{3}]-beta-casomorphin, amide, bovine; [DAla^{2}]-beta-casomorphin 1-4, amide, bovine; [DAla^{2}]-beta-casomorphin 1-5, bovine; [DAla^{2}]-beta-casomorphin 1-5, amide, bovine; [DAla^{2},Met^{5}]-beta-casomorphin 1-5, bovine; [DPro^{2}]-beta-casomorphin 1-5, amide, bovine; [DAla^{2}]-beta-casomorphin 1-6, bovine; [DPro^{2}]-beta-casomorphin 1-4, amide; [Des-Tyr^{1}]-beta-casomorphin, bovine; [DAla^{2,4},Tyr^{5}]-beta-casomorphin 1-5, amide, bovine; [DAla^{2}, (pCl)Phe^{3}]-beta-casomorphin, amide, bovine; [DAla^{2}]-beta-casomorphin 1-4, amide, bovine; [DAla^{2}]-beta-casomorphin 1-5, bovine; [DAla^{2}]-beta-casomorphin 1-5, amide, bovine; [DAla^{2},Met^{5}]-beta-casomorphin 1-5, bovine; [DPro^{2}]-beta-casomorphin 1-5, amide, bovine; [DAla^{2}]-beta-casomorphin 1-6, bovine; [DPro^{2}]-beta-casomorphin 1-4, amide; [Des-Tyr^{1}]-beta-casomorphin, bovine; and [Val^{3}]-beta-casomorphin 1-4, amide, bovine.

Chemotactic peptides including, but not limited to, defensin 1 (human) HNP-1 (human neutrophil peptide-1); and N-formyl-Met-Leu-Phe.

Cholecystokinin (CCK) peptides including, but not limited to, caerulein; cholecystokinin; cholecystokinin-pancreozymin; CCK-33, human; cholecystokinin octapeptide 1-4 (non-sulfated) (CCK 26-29, unsulfated); cholecystokinin octapeptide (CCK 26-33); cholecystokinin octapeptide (non-sulfated) (CCK 26-33, unsulfated); cholecystokinin heptapeptide (CCK 27-33); cholecystokinin tetrapeptide (CCK 30-33); CCK-33, porcine; CR 1 409, cholecystokinin antagonist; CCK flanking peptide (unsulfated); N-acetyl cholecystokinin, CCK 26-30, sulfated; N-acetyl cholecystokinin, CCK 26-31, sulfated; N-acetyl cholecystokinin, CCK 26-31, non-sulfated; prepro CCK fragment V-9-M; and proglumide.

Colony-stimulating factor peptides including, but not limited to, colony-stimulating factor (CSF); GMCSF; MCSF; and G-CSF.

Corticortropin releasing factor (CRF) peptides including, but not limited to, astressin; alpha-helical CRF 12-41; biotinyl-CRF, ovine; biotinyl-CRF, human, rat; CRF, bovine; CRF, human, rat; CRF, ovine; CRF, porcine; [Cys^{21}]-CRF, human, rat; CRF antagonist (alpha-helical CRF 9-41); CRF 6-33, human, rat; [$DPro^{5}$]-CRF, human, rat; [D-Phe^{12}, $Nle^{21,38}$]-CRF 12-41, human, rat; eosinophilotactic peptide; [$Met(0)^{21}$]-CRF, ovine; [Nle^{21},Tyr^{32}]-CRF, ovine; prepro CRF 125-151, human; sauvagine, frog; [Tyr^{0}]-CRF, human, rat; [Tyr^{0}]-CRF, ovine; [Tyr^{0}]-CRF 34-41, ovine; [Tyr^{0}]-urocortin; urocortin amide, human; urocortin, rat; urotensin I (Catostomus commersoni); urotensin II; and urotensin II (Rana ridibunda).

Cortistatin peptides including, but not limited to, cortistatin 29; cortistatin 29 (1-13); [Tyr^{0}]-cortistatin 29; pro-cortistatin 28-47; and pro-cortistatin 51-81.

Cytokine peptides including, but not limited to, tumor necrosis factor; and tumor necrosis factor-β (TNF-β).

Dermorphin peptides including, but not limited to, dermorphin and dermorphin analog 1-4.

Dynorphin peptides including, but not limited to, big dynorphin (prodynorphin 209-240), porcine; biotinyl-dynorphin A (biotinyl-prodynorphin 209-225); [$DAla^{2}$, $DArg^{6}$]-dynorphin A 1-13, porcine; [D-Ala^{2}]-dynorphin A, porcine; [D-Ala^{2}]-dynorphin A amide, porcine; [D-Ala^{2}]-dynorphin A 1-13, amide, porcine; [D-Ala^{2}]-dynorphin A 1-9, porcine; [$DArg^{6}$]-dynorphin A 1-13, porcine; [$DArg^{8}$]-dynorphin A 1-13, porcine; [Des-Tyr^{1}]-dynorphin A 1-8; [D-Pro^{10}]-dynorphin A 1-11, porcine; dynorphin A amide, porcine; dynorphin A 1-6, porcine; dynorphin A 1-7, porcine; dynorphin A 1-8, porcine; dynorphin A 1-9, porcine; dynorphin A 1-10, porcine; dynorphin A 1-10 amide, porcine; dynorphin A 1-11, porcine; dynorphin A 1-12, porcine; dynorphin A 1-13, porcine; dynorphin A 1-13 amide,

porcine; DAKLI (dynorphin A-analogue kappa ligand); DAKLI-biotin ([$Arg^{11,13}$]-dynorphin A (1-13)-Gly-NH(CH2)5NH-biotin); dynorphin A 2-17, porcine; dynorphin 2-17, amide, porcine; dynorphin A 2-12, porcine; dynorphin A 3-17, amide, porcine; dynorphin A 3-8, porcine; dynorphin A 3-13, porcine; dynorphin A 3-17, porcine; dynorphin A 7-17, porcine; dynorphin A 8-17, porcine; dynorphin A 6-17, porcine; dynorphin A 13-17, porcine; dynorphin A (prodynorphin 209-225), porcine; dynorphin B 1-9; [$MeTyr^1$, $MeArg^7$, D-Leu^8]-dynorphin 1-8 ethyl amide; [(nMe)Tyr^1] dynorphin A 1-13, amide, porcine; [Phe^7]-dynorphin A 1-7, porcine; [Phe^7]-dynorphin A 1-7, amide, porcine; and prodynorphin 228-256 (dynorphin B 29) (leumorphin), porcine.

Endorphin peptides including, but not limited to, alpha-neo-endorphin, porcine; beta-neo-endorphin; Ac-beta-endorphin, camel, bovine, ovine; Ac-beta-endorphin 1-27, camel, bovine, ovine; Ac-beta-endorphin, human; Ac-beta-endorphin 1-26, human; Ac-beta-endorphin 1-27, human; Ac-gamma-endorphin (Ac-beta-lipotropin 61-77); acetyl-alpha-endorphin; alpha-endorphin (beta-lipotropin 61-76); alpha-neo-endorphin analog; alpha-neo-endorphin 1-7; [Arg^8]-alpha-neo-endorphin 1-8; beta-endorphin (beta-lipotropin 61-91), camel, bovine, ovine; beta-endorphin 1-27, camel, bovine, ovine; beta-endorphin, equine; beta-endorphin (beta-lipotropin 61-91), human; beta-endorphin (1-5) + (16-31), human; beta-endorphin 1-26, human; beta-endorphin 1-27, human; beta-endorphin 6-31, human; beta-endorphin 18-31, human; beta-endorphin, porcine; beta-endorphin, rat; beta-lipotropin 1-10, porcine; beta-lipotropin 60-65; beta-lipotropin 61-64; beta-lipotropin 61-69; beta-lipotropin 88-91; biotinyl-beta-endorphin (biotinyl-beta-lipotropin 61-91); biocytin-beta-endorphin, human; gamma-endorphin (beta-lipotropin 61-77); [$DAla^2$]-alpha-neo-endorphin 1-2, amide; [$DAla^2$]-beta-lipotropin 61-69; [$DAla^2$]-gamma-endorphin; [Des-Tyr^1]-beta-endorphin, human; [Des-Tyr^1]-gamma-endorphin (beta-lipotropin 62-77); [Leu^5]-beta-endorphin, camel, bovine, ovine; [Met^5, Lys^6]-alpha-neo-endorphin 1-6; [Met^5, $Lys^{6,7}$]-alpha-neo-endorphin 1-7; and [Met^5, Lys^6, Arg^7]-alpha-neo-endorphin 1-7.

Endothelin peptides including, but not limited to, endothelin-1 (ET-1); endothelin-1[Biotin-Lys^9]; endothelin-1 (1-15), human; endothelin-1 (1-15), amide, human; Ac-endothelin-1 (16-21), human; Ac-[$DTrp^{16}$]-endothelin-1 (16-21), human; [$Ala^{3,11}$]-endothelin-1; [Dprl, Asp^{15}]-endothelin-1; [Ala^2]-endothelin-3, human; [Ala^{18}]-endothelin-1, human; [Asn^{18}]-endothelin-1, human; [Res-701-1]-endothelin B receptor antagonist; Suc-[Glu^9, $Ala^{11,15}$]-endothelin-1 (8-21), IRL-1620; endothelin-C-terminal hexapeptide; [D-Val^{22}]-big endothelin-1 (16-38), human; endothelin-2 (ET-2), human, canine; endothelin-3 (ET-3), human, rat, porcine, rabbit; biotinyl-endothelin-3 (biotinyl-ET-3); prepro-endothelin-1 (94-

109), porcine; BQ-518; BQ-610; BQ-788; endothelium-dependent relaxation antagonist; FR139317; IRL-1038; JKC-30 1; JKC-302; PD-145065; PD 142893; sarafotoxin S6a (atractaspis engaddensis); sarafotoxin S6b (atractaspis engaddensis); sarafotoxin S6c (atractaspis engaddensis); [Lys^4]-sarafotoxin S6c; sarafotoxin S6d; big endothelin-1, human; biotinyl-big endothelin-1, human; big endothelin-1 (1-39), porcine; big endothelin-3 (22-41), amide, human; big endothelin-1 (22-39), rat; big endothelin-1 (1-39), bovine; big endothelin-1 (22-39), bovine; big endothelin-1 (19-38), human; big endothelin-1 (22-38), human; big endothelin-2, human; big endothelin-2 (22-37), human; big endothelin-3, human; big endothelin-1, porcine; big endothelin-1 (22-39) (prepro-endothelin-1 (74-91)); big endothelin-1, rat; big endothelin-2 (1-38), human; big endothelin-2 (22-38), human; big endothelin-3, rat; biotinyl-big endothelin-1, human; and [Tyr^{123}]-prepro-endothelin (110-130), amide, human.

ETa receptor antagonist peptides including, but not limited to, [BQ-123]; [BE18257B]; [BE-18257A]/[W-7338A]; [BQ-485]; FR139317; PD-151242; and TTA-386.

ETb receptor antagonist peptides including, but not limited to, [BQ-3020]; [RES-701-3]; and [IRL-1720]

Enkephalin peptides including, but not limited to, adrenorphin, free acid; amidorphin (proenkephalin A (104-129)-NH2), bovine; BAM-12P (bovine adrenal medulla dodecapeptide); BAM-22P (bovine adrenal medulla docosapeptide); benzoyl-Phe- Ala-Arg; enkephalin; [D-Ala^2, D-Leu^5]-enkephalin; [D-Ala^2, D-Met^5]-enkephalin; [DAla^2]-Leu-enkephalin, amide; [DAla^2,Leu^5,Arg^6]-enkephalin; [Des-Tyr^1,D$Pen^{2,5}$]-enkephalin; [Des-Tyr^1,DPen^2,Pen^5]-enkephalin; [Des-Tyr^1]-Leu-enkephalin; [D-$Pen^{2,5}$]-enkephalin; [DPen^2, Pen^5]-enkephalin; enkephalinase substrate; [D-Pen^2, pCI-Phe^4, D-Pen^5]-enkephalin; Leu-enkephalin; Leu-enkephalin, amide; biotinyl-Leu-enkephalin; [D-Ala^2]-Leu-enkephalin; [D-Ser^2]-Leu-enkephalin-Thr (delta-receptor peptide) (DSLET); [D-Thr^2]-Leu-enkephalin-Thr (DTLET); [Lys^6]-Leu-enkephalin; [Met^5,Arg^6]-enkephalin; [Met^5,Arg^6]-enkephalin-Arg; [Met^5,Arg^6,Phe^7]-enkephalin, amide; Met-enkephalin; biotinyl-Met-enkephalin; [D-Ala^2]-Met-enkephalin; [D-Ala^2]-Met-enkephalin, amide; Met-enkephalin-Arg-Phe; Met-enkephalin, amide; [Ala^2]-Met-enkephalin, amide; [DMet^2,Pro^5]-enkephalin, amide; [DTrp^2]-Met-enkephalin, amide, metorphinamide (adrenorphin); peptide B, bovine; 3200-Dalton adrenal peptide E, bovine; peptide F, bovine; preproenkephalin B 186-204, human; spinorphin, bovine; and thiorphan (D, L, 3-mercapto-2-benzylpropanoyl-glycine).

Fibronectin peptides including, but not limited to platelet factor-4 (58-70), human; echistatin (Echis carinatus); E, P ,L selectin conserved region; fibronectin analog;

fibronectin-binding protein; fibrinopeptide A, human; [Tyr^0]-fibrinopeptide A, human; fibrinopeptide B, human; [Glu^1]-fibrinopeptide B, human; [Tyr^{15}]-fibrinopeptide B, human; fibrinogen beta-chain fragment of 24-42; fibrinogen binding inhibitor peptide; fibronectin related peptide (collagen binding fragment); fibrinolysis inhibiting factor; FN-C/H-1 (fibronectin heparin-binding fragment); FN-C/H-V (fibronectin heparin-binding fragment); heparin-binding peptide; laminin penta peptide, amide; Leu-Asp-Val-NH2 (LDV-NH2), human, bovine, rat, chicken; necrofibrin, human; necrofibrin, rat; and platelet membrane glycoprotein IIB peptide 296-306.

Galanin peptides including, but not limited to, galanin, human; galanin 1-19, human; preprogalanin 1-30, human; preprogalanin 65-88, human; preprogalanin 89-123, human; galanin, porcine; galanin 1-16, porcine, rat; galanin, rat; biotinyl-galanin, rat; preprogalanin 28-67, rat; galanin 1-13-bradykinin 2-9, amide; M40, galanin 1-13-Pro-Pro-(Ala-Leu) 2-Ala-amide; C7, galanin 1-13-spantide-amide; GMAP 1-41, amide; GMAP 16-41, amide; GMAP 25-41, amide; galantide; and entero-kassinin.

Gastrin peptides including, but not limited to, gastrin, chicken; gastric inhibitory peptide (GIP), human; gastrin I, human; biotinyl-gastrin I, human; big gastrin-1, human; gastrin releasing peptide, human; gastrin releasing peptide 1-16, human; gastric inhibitory polypeptide (GIP), porcine; gastrin releasing peptide, porcine; biotinyl-gastrin releasing peptide, porcine; gastrin releasing peptide 14-27, porcine, human; little gastrin, rat; pentagastrin; gastric inhibitory peptide 1-30, porcine; gastric inhibitory peptide 1-30, amide, porcine; [Tyr^0]-gastric inhibitory peptide 23-42, human; and gastric inhibitory peptide, rat.

Glucagon peptides including, but not limited to, [Des-His^1,Glu^9]-glucagon, extendin-4, glucagon, human; biotinyl-glucagon, human; glucagon 19-29, human; glucagon 22-29, human; Des-His^1-[Glu^9]-glucagon, amide; glucagon-like peptide 1, amide (preproglucagon 72-107, amide); glucagon-like peptide 1 (preproglucagon 72-108), human; glucagon-like peptide 1 (7-36) (preproglucagon 78-107, amide); glucagon-like peptide II, rat; biotinyl-glucagon-like peptide-1 (7-36) (biotinyl-preproglucagon 78-107, amide); glucagon-like peptide 2 (preproglucagon 126-159), human; oxyntomodulin/glucagon 37; and valosin (peptide VQY), porcine.

Gn-RH associated peptides (GAP) including, but not limited to, Gn-RH associated peptide 25-53, human; Gn-RH associated peptide 1-24, human; Gn-RH associated peptide 1-13, human; Gn-RH associated peptide 1-13, rat; gonadotropin releasing peptide, follicular, human; [Tyr^0]-GAP ([Tyr^0]-Gn-RH Precursor Peptide 14-69), human; and proopiomelanocortin (POMC) precursor 27-52, porcine.

Growth factor peptides including, but not limited to, cell growth factors; epidermal growth factors; tumor growth factor; alpha-TGF; beta-TF; alpha-TGF 34-43, rat; EGF, human; acidic fibroblast growth factor; basic fibroblast growth factor; basic fibroblast growth factor 13-18; basic fibroblast growth factor 120-125; brain derived acidic fibroblast growth factor 1-11; brain derived basic fibroblast growth factor 1-24; brain derived acidic fibroblast growth factor 102-111; [Cys(Acm^{20,31})]-epidermal growth factor 20-31; epidermal growth factor receptor peptide 985-996; insulin-like growth factor (IGF)-I, chicken; IGF-I, rat; IGF-I, human; Des (1-3) IGF-I, human; R3 IGF-I, human; R3 IGF-I, human; long R3 IGF-I, human; adjuvant peptide analog; anorexigenic peptide; Des (1-6) IGF-II, human; R6 IGF-II, human; IGF-I analogue; IGF I (24-41); IGF I (57-70); IGF I (30-41); IGF II; IGF II (33-40); [Tyr^{0}]-IGF II (33-40); liver cell growth factor; midkine; midkine 60-121, human; N-acetyl, alpha-TGF 34-43, methyl ester, rat; nerve growth factor (NGF), mouse; platelet-derived growth factor; platelet-derived growth factor antagonist; transforming growth factor-alpha, human; and transforming growth factor-I, rat.

Growth hormone peptides including, but not limited to, growth hormone (hGH), human; growth hormone 1-43, human; growth hormone 6-13, human; growth hormone releasing factor, human; growth hormone releasing factor, bovine; growth hormone releasing factor, porcine; growth hormone releasing factor 1-29, amide, rat; growth hormone pro-releasing factor, human; biotinyl-growth hormone releasing factor, human; growth hormone releasing factor 1-29, amide, human; [D-Ala^{2}]-growth hormone releasing factor 1-29, amide, human; [N-Ac-Tyr^{1}, D-Arg^{2}]-GRF 1-29, amide; [His^{1}, Nle^{27}]-growth hormone releasing factor 1-32, amide; growth hormone releasing factor 1-37, human; growth hormone releasing factor 1-40, human; growth hormone releasing factor 1-40, amide, human; growth hormone releasing factor 30-44, amide, human; growth hormone releasing factor, mouse; growth hormone releasing factor, ovine; growth hormone releasing factor, rat; biotinyl- growth hormone releasing factor, rat; GHRP-6 ([His^{1}, Lys^{6}]-GHRP); hexarelin (growth hormone releasing hexapeptide); and [D-Lys^{3}]-GHRP-6.

GTP-binding protein fragment peptides including, but not limited to, [Arg^{8}]-GTP-binding protein fragment, Gs alpha; GTP-binding protein fragment, G beta; GTP-binding protein fragment, GAlpha; GTP-binding protein fragment, Go Alpha; GTP-binding protein fragment, Gs Alpha; and GTP-binding protein fragment, G Alpha i2.

Guanylin peptides including, but not limited to, guanylin, human; guanylin, rat; and uroguanylin.

Inhibin peptides including, but not limited to, inhibin, bovine; inhibin, alpha-subunit 1-32, human; [Tyr^0]-inhibin, alpha-subunit 1-32, human; seminal plasma inhibin-like peptide, human; [Tyr^0]-seminal plasma inhibin-like peptide, human; inhibin, alpha-subunit 1-32, porcine; and [Tyr^0]-inhibin, alpha-subunit 1-32, porcine.

Insulin peptides including, but not limited to, insulin, human; insulin, porcine; IGF-I, human; insulin-like growth factor II (69-84); pro-insulin-like growth factor II (68-102), human; pro-insulin-like growth factor II (105-128), human; [Asp^{B28}]-insulin, human; [Lys^{B28}]-insulin, human; [Leu^{B28}]-insulin, human; [Val^{B28}]-insulin, human; [Ala^{B28}]-insulin, human; [Asp^{B28}, Pro^{B29}]-insulin, human; [Lys^{B28}, Pro^{B29}]-insulin, human; [Leu^{B28}, Pro^{B29}]-insulin, human; [Val^{B28}, Pro^{B29}]-insulin, human; [Ala^{B28}, Pro^{B29}]-insulin, human; [Gly^{A21}]-insulin, human; [Gly^{A21} Gln^{B3}]-insulin, human; [Ala^{A21}]-insulin, human; [Ala^{A21} Gln^{B3}]-insulin, human; [Gln^{B3}]-insulin, human; [Gln^{B30}]-insulin, human; [Gly^{A21} Glu^{B30}]-insulin, human; [Gly^{A21} Gln^{B3} Glu^{B30}]-insulin, human; [Gln^{B3} Glu^{B30}]-insulin, human; B22-B30 insulin, human; B23-B30 insulin, human; B25-B30 insulin, human; B26-B30 insulin, human; B27-B30 insulin, human; B29-B30 insulin, human; the A chain of human insulin, and the B chain of human insulin.

Interleukin peptides including, but not limited to, interleukin-1 beta 165-181, rat; and interleukin-8 (IL-8, CINC/gro), rat.

Laminin peptides including, but not limited to, laminin; alpha1 (I)-CB3 435-438, rat; and laminin binding inhibitor.

Leptin peptides including, but not limited to, leptin 93-105, human; leptin 22-56, rat; Tyr-leptin 26-39, human; and leptin 116-130, amide, mouse.

Leucokinin peptides including, but not limited to, leucomyosuppressin (LMS); leucopyrokinin (LPK); leucokinin I; leucokinin II; leucokinin III; leucokinin IV; leucokinin VI; leucokinin VII; and leucokinin VIII.

Luteinizing hormone-releasing hormone peptides including, but not limited to, antide; Gn-RH II, chicken; luteinizing hormone-releasing hormone (LH-RH) (GnRH); biotinyl-LH-RH; cetrorelix (D-20761); [D-Ala^6]-LH-RH; [Gln^8]-LH-RH (Chicken LH-RH); [DLeu^6, Val^7] LH-RH 1-9, ethyl amide; [D-Lys^6]-LH-RH; [D-Phe^2, Pro^3, D-Phe^6]-LH-RH; [DPhe^2, DAla^6] LH-RH; [Des-Gly^{10}]-LH-RH, ethyl amide; [D-Ala^6, Des-Gly^{10}]-LH-RH, ethyl amide; [DTrp^6]-LH-RH, ethyl amide; [D-Trp^6, Des-Gly^{10}]-LH-RH, ethyl amide (Deslorelin); [DSer(But)^6, Des-Gly^{10}]-LH-RH, ethyl amide; ethyl amide; leuprolide; LH-RH 4-10; LH-RH 7-10; LH-RH, free acid; LH-RH, lamprey; LH-RH, salmon; [Lys^8]-LH-RH; [Trp^7,Leu^8] LH-RH, free acid; and [(t-Bu)DSer^6, (Aza)Gly^{10}]-LH-RH.

Mastoparan peptides including, but not limited to, mastoparan; mas7; mas8; mas17; and mastoparan X.

Mast cell degranulating peptides including, but not limited to, mast cell degranulating peptide HR-1; and mast cell degranulating peptide HR-2.

Melanocyte stimulating hormone (MSH) peptides including, but not limited to, [Ac-Cys^4,$DPhe^7$,Cys^{10}] alpha-MSH 4-13, amide; alpha-melanocyte stimulating hormone; alpha-MSH, free acid; beta-MSH, porcine; biotinyl-alpha-melanocyte stimulating hormone; biotinyl-[Nle^4, D-Phe^7] alpha-melanocyte stimulating hormone; [Des -Acetyl]-alpha-MSH; [$DPhe^7$]-alpha-MSH, amide; gamma-1-MSH, amide; [Lys^0]-gamma-1-MSH, amide; MSH release inhibiting factor, amide; [Nle^4]-alpha-MSH, amide; [Nle^4, D-Phe^7]-alpha-MSH; N-Acetyl, [Nle^4,$DPhe^7$] alpha-MSH 4-10, amide; beta-MSH, human; and gamma-MSH.

Morphiceptin peptides including, but not limited to, morphiceptin (beta-casomorphin 1-4 amide); [D-Pro^4]-morphiceptin; and [N-$MePhe^3$,D-Pro^4]-morphiceptin.

Motilin peptides including, but not limited to, motilin, canine; motilin, porcine; biotinyl-motilin, porcine; and [Leu^{13}]-motilin, porcine.

Neuro-peptides including, but not limited to, Ac-Asp-Glu; achatina cardio-excitatory peptide-1 (ACEP-1) (Achatina fulica); adipokinetic hormone (AKH) (Locust); adipokinetic hormone (Heliothis zea and Manduca sexta); alytesin; Tabanus atratus adipokinetic hormone (Taa-AKH); adipokinetic hormone II (Locusta migratoria); adipokinetic hormone II (Schistocera gregaria); adipokinetic hormone III (AKH-3); adipokinetic hormone G (AKH-G) (Gryllus bimaculatus); allatotropin (AT) (Manduca sexta); allatotropin 6-13 (Manduca sexta); APGW amide (Lymnaea stagnalis); buccalin; cerebellin; [Des-Ser^1]-cerebellin; corazonin (American Cockroach Periplaneta americana); crustacean cardioactive peptide (CCAP); crustacean erythrophore; DF2 (Procambarus clarkii); diazepam-binding inhibitor fragment, human; diazepam binding inhibitor fragment (ODN); eledoisin related peptide; FMRF amide (molluscan cardioexcitatory neuro-peptide); Gly-Pro-Glu (GPE), human; granuliberin R; head activator neuropeptide; [His^7]-corazonin; stick insect hypertrehalosaemic factor II; Tabanus atratus hypotrehalosemic hormone (Taa-HoTH); isoguvacine hydrochloride; bicuculline methiodide; piperidine-4-sulphonic acid; joining peptide of proopiomelanocortin (POMC), bovine; joining peptide, rat; KSAYMRF amide (P. redivivus); kassinin; kinetensin; levitide; litorin; LUQ 81-91 (Aplysia californica); LUQ 83-91 (Aplysia californica); myoactive peptide I (Periplanetin CC-1) (Neuro-hormone D); myoactive peptide II (Periplanetin CC-2); myomodulin; neuron specific peptide; neuron specific enolase 404-443, rat; neuropeptide FF; neuropeptide K, porcine; NEI (prepro-MCH

131-143) neuropeptide, rat; NGE (prepro-MCH 110-128) neuropeptide, rat; NFl (Procambarus clarkii); PBAN-l (Bombyx mori); Hez-PBAN (Heliothis zea); SCPB (cardioactive peptide from aplysia); secretoneurin, rat; uperolein; urechistachykinin I; urechistachykinin II; xenopsin-related peptide I; xenopsin-related peptide II; pedal peptide (Pep), aplysia; peptide Fl, lobster; phyllomedusin; polistes mastoparan; proctolin; ranatensin; Ro I (Lubber Grasshopper, Romalea microptera); Ro II (Lubber Grasshopper, Romalea microptera); SALMF amide 1 (S1); SALMF amide 2 (S2); and SCPA.

Neuropeptide Y (NPY) peptides including, but not limited to, [Leu^{31},Pro^{34}]-neuropeptide Y, human; neuropeptide F (Moniezia expansa); B1BP3226 NPY antagonist; Bis (31/31') {[Cys^{31}, Trp^{32}, Nva^{34}] NPY 31-36}; neuropeptide Y, human, rat; neuropeptide Y 1-24 amide, human; biotinyl-neuropeptide Y; [D-Tyr^{27,36}, D-Thr^{32}]-NPY 27-36; Des 10-17 (cyclo 7-21) [Cys^{7,21}, Pro^{34}]-NPY; C2-NPY; [Leu^{31}, Pro^{34}] neuropeptide Y, human; neuropeptide Y, free acid, human; neuropeptide Y, free acid, porcine; prepro NPY 68-97, human; N-acetyl-[Leu^{28}, Leu^{31}] NPY 24-36; neuropeptide Y, porcine; [D-Trp^{32}]-neuropeptide Y, porcine; [D-Trp^{32}] NPY 1-36, human; [Leu^{17},DTrp^{32}] neuropeptide Y, human; [Leu^{31}, Pro^{34}]-NPY, porcine; NPY 2-36, porcine; NPY 3-36, human; NPY 3-36, porcine; NPY 13-36, human; NPY 13-36, porcine; NPY 16-36. porcine; NPY 18-36, porcine; NPY 20-36; NFY 22-36; NPY 26-36; [Pro^{34}]-NPY 1-36, human; [Pro^{34}]-neuropeptide Y, porcine; PYX-1; PYX-2; T4-[NPY(33-36)]4; and Tyr(OMe)^{21}]-neuropeptide Y, human.

Neurotropic factor peptides including, but not limited to, glial derived neurotropic factor (GDNF); brain derived neurotropic factor (BDNF); and ciliary neurotropic factor (CNTF).

Orexin peptides including, but not limited to, orexin A; orexin B, human; orexin B, rat, mouse.

Opioid peptides including, but not limited to, alpha-casein fragment 90-95; BAM-18P; casomokinin L; casoxin D; crystalline; DALDA; dermenkephalin (deltorphin) (Phylomedusa sauvagei); [D-Ala^{2}]-deltorphin I; [D-Ala^{2}]-deltorphin II; endomorphin-l; endomorphin-2; kyotorphin; [DArg^{2}]-kyotorphin; morphin tolerance peptide; morphine modulating peptide, C-terminal fragment; morphine modulating neuropeptide (A-18-F-NH2); nociceptin [orphanin FQ] (ORL1 agonist); TIPP; Tyr-MIF-l; Tyr-W-MIF-1; valorphin; LW-hemorphin-6, human; Leu-valorphin-Arg; and Z-Pro-D-Leu.

Oxytocin peptides including, but not limited to, [Asu^{6}]-oxytocin; oxytocin; biotinyl-oxytocin; [Thr^{4}, Gly^{7}]-oxytocin; and tocinoic acid ([Ile^{3}]-pressinoic acid).

PACAP (pituitary adenylating cyclase activating peptide) peptides including, but not limited to, PACAP 1-27, human, ovine, rat; PACAP (1-27)-Gly-Lys-Arg-NH2, human; [Des-Gln^{16}]-PACAP 6-27, human, ovine, rat; PACAP38, frog; PACAP27-NH2, human, ovine, rat; biotinyl-PACAP27-NH2, human, ovine, rat; PACAP 6-27, human, ovine, rat; PACAP38, human, ovine, rat; biotinyl-PACAP38, human, ovine, rat; PACAP 6-38, human, ovine, rat; PACAP27-NH2, human, ovine, rat; biotinyl-PACAP27-NH2, human, ovine, rat; PACAP 6-27, human, ovine, rat; PACAP38, human, ovine, rat; biotinyl-PACAP38, human, ovine, rat; PACAP 6-38, human, ovine, rat; PACAP38 16-38, human, ovine, rat; PACAP38 31-38, human, ovine, rat; PACAP38 31-38, human, ovine, rat; PACAP-related peptide (PRP), human; and PACAP-related peptide (PRP), rat.

Pancreastatin peptides including, but not limited to, chromostatin, bovine; pancreastatin (hPST-52) (chromogranin A 250-301, amide); pancreastatin 24-52 (hPST-29), human; chromogranin A 286-301, amide, human; pancreastatin, porcine; biotinyl-pancreastatin, porcine; [Nle^8]-pancreastatin, porcine; [Tyr^0,Nle^8]-pancreastatin, porcine; [Tyr^0]-pancreastatin, porcine; parastatin 1-19 (chromogranin A 347–365), porcine; pancreastatin (chromogranin A 264-314-amide, rat; biotinyl-pancreastatin (biotinyl-chromogranin A 264-314-amide; [Tyr^0]-pancreastatin, rat; pancreastatin 26-51, rat; and pancreastatin 33-49, porcine.

Pancreatic polypeptides including, but not limited to, pancreatic polypeptide, avian; pancreatic polypeptide, human; C-fragment pancreatic polypeptide acid, human; C-fragment pancreatic polypeptide amide, human; pancreatic polypeptide (Rana temporaria); pancreatic polypeptide, rat; and pancreatic polypeptide, salmon.

Parathyroid hormone peptides including, but not limited to, [Asp^{76}]-parathyroid hormone 39-84, human; [Asp^{76}]-parathyroid hormone 53-84, human; [Asn^{76}]-parathyroid hormone 1-84, hormone; [Asn^{76}]-parathyroid hormone 64-84, human; [Asn^8, Leu^{18}]-parathyroid hormone 1-34, human; [$Cys^{5,28}$]-parathyroid hormone 1-34, human; hypercalcemia malignancy factor 1-40; [Leu^{18}]-parathyroid hormone 1-34, human; [Lys(biotinyl)^{13}, $Nle^{8,18}$, Tyr^{34}]-parathyroid hormone 1-34 amide; [$Nle^{8,18}$, Tyr^{34}]-parathyroid hormone 1-34 amide; [$Nle^{8,18}$, Tyr^{34}]-parathyroid hormone 3-34 amide, bovine; [$Nle^{8,18}$, Tyr^{34}]-parathyroid hormone 1-34, human; [$Nle^{8,18}$, Tyr^{34}]-parathyroid hormone 1-34 amide, human; [$Nle^{8,18}$, Tyr^{34}]-parathyroid hormone 3-34 amide, human; [$Nle^{8,18}$, Tyr^{34}]- parathyroid hormone 7-34 amide, bovine; [$Nle^{8,21}$, Tyr^{34}]-parathyroid hormone 1-34 amide, rat; parathyroid hormone 44-68, human; parathyroid hormone 1-34, bovine; parathyroid hormone 3-34, bovine; parathyroid hormone 1-31 amide, human; parathyroid hormone 1-34, human;

parathyroid hormone 13-34, human; parathyroid hormone 1-34, rat; parathyroid hormone 1-38, human; parathyroid hormone 1-44, human; parathyroid hormone 28-48, human; parathyroid hormone 39-68, human; parathyroid hormone 39-84, human; parathyroid hormone 53-84, human; parathyroid hormone 69-84, human; parathyroid hormone 70-84, human; [Pro^{34}]-peptide YY (PYY), human; [Tyr^{0}]-hypercalcemia malignancy factor 1-40; [Tyr^{0}]-parathyroid hormone 1-44, human; [Tyr^{0}]-parathyroid hormone 1-34, human; [Tyr^{1}]-parathyroid hormone 1-34, human; [Tyr^{27}]-parathyroid hormone 27-48, human; [Tyr^{34}]-parathyroid hormone 7-34 amide, bovine; [Tyr^{43}]-parathyroid hormone 43-68, human; [Tyr^{52}, Asn^{76}]-parathyroid hormone 52-84, human; and [Tyr^{63}]-parathyroid hormone 63-84, human.

Parathyroid hormone (PTH)-related peptides including, but not limited to, PTHrP ([Tyr^{36}]-PTHrP 1-36 amide), chicken; hHCF-(1-34)-NH2 (humoral hypercalcemic factor), human; PTH-related protein 1-34, human; biotinyl-PTH-related protein 1-34, human; [Tyr^{0}]-PTH-related protein 1-34, human; [Tyr^{34}]-PTH-related protein 1-34 amide, human; PTH-related protein 1-37, human; PTH-related protein 7-34 amide, human; PTH-related protein 38-64 amide, human; PTH-related protein 67-86 amide, human; PTH-related protein 107-111, human, rat, mouse; PTH-related protein 107-111 free acid; PTH-related protein 107-138, human; and PTH-related protein 109-111, human.

Peptide T peptides including, but not limited to, peptide T; [D–Ala^{1}]-peptide T; and [D–Ala^{1}]-peptide T amide.

Prolactin-releasing peptides including, but not limited to, prolactin-releasing peptide 31, human; prolactin-releasing peptide 20, human; prolactin-releasing peptide 31, rat; prolactin-releasing peptide 20, rat; prolactin-releasing peptide 31, bovine; and prolactin-releasing peptide 20, bovine.

Peptide YY (PYY) peptides including, but not limited to, PYY, human; PYY 3-36, human; biotinyl-PYY, human; PYY, porcine, rat; and [Leu^{31}, Pro^{34}]-PYY, human.

Renin substrate peptides including, but not limited to, acetyl, angiotensinogen 1-14, human; angiotensinogen 1-14, porcine; renin substrate tetradecapeptide, rat; [Cys^{8}]-renin substrate tetradecapeptide, rat; [Leu^{8}]-renin substrate tetradecapeptide, rat; and [Val^{8}]-renin substrate tetradecapeptide, rat.

Secretin peptides including, but not limited to, secretin, canine; secretin, chicken; secretin, human; biotinyl-secretin, human; secretin, porcine; and secretin, rat.

Somatostatin (GIF) peptides including, but not limited to, BIM-23027; biotinyl-somatostatin; biotinylated cortistatin 17, human; cortistatin 14, rat; cortistatin 17, human; [Tyr^{0}]-cortistatin 17, human; cortistatin 29, rat; [D-Trp^{8}]-somatostatin; [$DTrp^{8}$,$DCys^{14}$]-

somatostatin; [DTrp^{8},Tyr^{11}]-somatostatin; [D-Trp^{11}]-somatostatin; NTB (Naltriben); [Nle^{8}]-somatostatin 1-28; octreotide (SMS 201-995); prosomatostatin 1-32, porcine; [Tyr^{0}]-somatostatin; [Tyr^{1}]-somatostatin; [Tyr^{1}]-somatostatin 28 (1-14); [Tyr^{11}]-somatostatin; [Tyr^{0}, D-Trp^{8}]-somatostatin; somatostatin; somatostatin antagonist; somatostatin-25; somatostatin-28; somatostatin 28 (1-12); biotinyl-somatostatin-28; [Tyr^{0}]-somatostatin-28; [Leu^{8}, D-Trp^{22}, Tyr^{25}]-somatostatin-28; biotinyl-[Leu^{8}, D-Trp^{22}, Tyr^{25}]-somatostatin-28; somatostatin-28 (1-14); and somatostatin analog, RC-160.

Substance P peptides including, but not limited to, G protein antagonist-2; Ac-[Arg^{6}, Sar^{9}, Met(02)^{11}]-substance P 6-11; [Arg^{3}]-substance P; Ac-Trp-3,5-bis(trifluoromethyl) benzyl ester; Ac-[Arg^{6}, Sar^{9}, Met(O2)^{11}]-substance P 6-11; [D-Ala^{4}]-substance P 4-11; [Tyr^{6}, D-Phe^{7}, D-His^{9}]-substance P 6-11 (sendide); biotinyl-substance P; biotinyl-NTE[Arg^{3}]-substance P; [Tyr^{8}]-substance P; [Sar^{9}, Met(O2)^{11}]-substance P; [D-Pro^{2}, D-Trp^{7,9}]-substance P; [D-Pro^{4}, 0-Trp^{7,9}]-substance P 4-11; substance P 4-11; [DTrp^{2,7,9}]-substance P; [(Dehydro)Pro^{2,4}, Pro^{9}]-substance P; [Dehydro-Pro^{4}]-substance P 4-11; [Glp^{5},(Me)Phe^{8},Sar^{9}]-substance P 5-11; [Glp^{5},Sar^{9}]-substance P 5-11; [Glp^{5}]-substance P 5-11; hepta-substance P (substance P 5-11); hexa-substance P(substance P 6-11); [MePhe^{8},Sar^{9}]-substance P; [Nle^{11}]-substance P; Octa-substance P(substance P 4-11); [pGlu^{1}]-hexa-substance P ([pGlu^{6}]-substance P 6-11); [pGlu^{6}, D-Pro^{9}]-substance P 6-11; [(pNO2)Phe^{7}Nle^{11}]-substance P; penta-substance P (substance P 7-11); [Pro^{9}]-substance P; GR73632, substance P 7-11; [Sar^{4}]-substance P 4-11; [Sar^{9}]-substance P; septide ([pGlu^{6}, Pro^{9}]-substance P 6-11); spantide I; spantide II; substance P; substance P, cod; substance P, trout; substance P antagonist; substance P-Gly-Lys-Arg; substance P 1-4; substance P 1-6; substance P 1-7; substance P 1-9; deca-substance P (substance P 2-11); nona-substance P (substance P 3-11); substance P tetrapeptide (substance P 8-11); substance P tripeptide (substance P 9-11); substance P, free acid; substance P methyl ester; and [Tyr^{8},Nle^{11}] substance P.

Tachykinin peptides including, but not limited to, [Ala^{5}, beta-Ala^{8}] neurokinin A 4-10; eledoisin; locustatachykinin I (Lom-TK-I) (Locusta migratoria); locustatachykinin II (Lom-TK-II) (Locusta migratoria); neurokinin A 4-10; neurokinin A (neuromedin L, substance K); neurokinin A, cod and trout; biotinyl-neurokinin A (biotinyl-neuromedin L, biotinyl-substance K); [Tyr^{0}]-neurokinin A; [Tyr^{6}]-substance K; FR64349; [Lys^{3}, Gly^{8}-(R)-gamma-lactam-Leu^{9}]-neurokinin A 3-10; GR83074; GR87389; GR94800; [Beta-Ala^{8}]-neurokinin A 4-10; [Nle^{10}]-neurokinin A 4-10; [Trp^{7}, beta–Ala^{8}]-neurokinin A 4-10; neurokinin B (neuromedin K); biotinyl-neurokinin B (biotinyl-neuromedin K); [MePhe^{7}]-neurokinin B; [Pro^{7}]-neurokinin B; [Tyr^{0}]-neurokinin B; neuromedin B, porcine; biotinyl-

neuromedin B, porcine; neuromedin B-30, porcine; neuromedin B-32, porcine; neuromedin B receptor antagonist; neuromedin C, porcine; neuromedin N, porcine; neuromedin (U-8), porcine; neuromedin (U-25), porcine; neuromedin U, rat; neuropeptide-gamma (gamma-preprotachykinin 72-92); PG-KII; phyllolitorin; [Leu^8]-phyllolitorin (Phyllomedusa sauvagei); physalaemin; physalaemin 1-11; scyliorhinin II, amide, dogfish; senktide, selective neurokinin B receptor peptide; [Ser^2]-neuromedin C; beta-preprotachykinin 69-91, human; beta-preprotachykinin 111-129, human; tachyplesin I; xenopsin; and xenopsin 25 (xenin 25), human.

Thyrotropin-releasing hormone (TRH) peptides including, but not limited to, biotinyl-thyrotropin-releasing hormone; [Glu^1]-TRH; His-Pro-diketopiperazine; [3-Me-His^2]-TRH; pGlu-Gln-Pro-amide; pGlu-His; [Phe^2]-TRH; prepro TRH 53-74; prepro TRH 83-106; prepro-TRH 160-169 (Ps4, TRH-potentiating peptide); prepro-TRH 178-199; thyrotropin-releasing hormone (TRH); TRH, free acid; TRH-SH Pro; and TRH precursor peptide.

Toxin peptides including, but not limited to, omega-agatoxin TK; agelenin, (spider, Agelena opulenta); apamin (honeybee, Apis mellifera); calcicudine (CaC) (green mamba, Dedroaspis angusticeps); calciseptine (black mamba, Dendroaspis polylepis polylepis); charybdotoxin (ChTX) (scorpion, Leiurus quinquestriatus var. hebraeus); chlorotoxin; conotoxin GI (marine snail, Conus geographus); conotoxin GS (marine snail, Conus geographus); conotoxin MI (Marine Conus magus); alpha-conotoxin EI, Conus ermineus; alpha-conotoxin SIA; alpha-conotoxin Iml; alpha-conotoxin SI (cone snail, Conus striatus); micro-conotoxin GIIIB (marine snail, Conus geographus); omega-conotoxin GVIA (marine snail, Conus geographus); omega-conotoxin MVIIA (Conus magus); omega-conotoxin MVIIC (Conus magus); omega-conotoxin SVIB (cone snail, Conus striatus); endotoxin inhibitor; geographutoxin I (GTX-I) (μ-Conotoxin GIIIA); iberiotoxin (IbTX) (scorpion, Buthus tamulus); kaliotoxin 1-37; kaliotoxin (scorpion, Androct-onus mauretanicus mauretanicus); mast cell-degranulating peptide (MCD-peptide, peptide 401); margatoxin (MgTX) (scorpion, Centruriodes Margaritatus); neurotoxin NSTX-3 (pupua new guinean spider, Nephilia maculata); PLTX-II (spider, Plectreurys tristes); scyllatoxin (leiurotoxin I); and stichodactyla toxin (ShK).

Vasoactive intestinal peptides (VIP/PHI) including, but not limited to, VIP, human, porcine, rat, ovine; VIP-Gly-Lys-Arg-NH2; biotinyl-PHI (biotinyl-PHI-27), porcine; [Glp^{16}] VIP 16-28, porcine; PHI (PHI-27), porcine; PHI (PHI-27), rat; PHM-27 (PHI), human; prepro VIP 81-122, human; preproVIP/PHM 111-122; prepro VIP/PHM 156-170; biotinyl-PHM-27 (biotinyl-PHI), human; vasoactive intestinal contractor (endothelin-beta); vasoactive

intestinal octacosa-peptide, chicken; vasoactive intestinal peptide, guinea pig; biotinyl-VIP, human, porcine, rat; vasoactive intestinal peptide 1-12, human, porcine, rat; vasoactive intestinal peptide 10-28, human, porcine, rat; vasoactive intestinal peptide 11-28, human, porcine, rat, ovine; vasoactive intestinal peptide (cod, Gadus morhua); vasoactive intestinal peptide 6-28; vasoactive intestinal peptide antagonist; vasoactive intestinal peptide antagonist ([Ac-Tyr^1, D-Phe^2]-GHRF 1-29 amide); vasoactive intestinal peptide receptor antagonist (4-Cl-D-Phe^6, Leu^{17}]-VIP); and vasoactive intestinal peptide receptor binding inhibitor, L-8-K.

Vasopressin (ADH) peptides including, but not limited to, vasopressin; [Asu^{1,6},Arg^8]-vasopressin; vasotocin; [Asu^{1,6},Arg^8]-vasotocin; [Lys^8]-vasopressin; pressinoic acid; [Arg^8]-desamino vasopressin desglycinamide; [Arg^8]-vasopressin (AVP); [Arg^8]-vasopressin desglycinamide; biotinyl-[Arg^8]-vasopressin (biotinyl-AVP); [D-Arg^8]-vasopressin; desamino-[Arg^8]-vasopressin; desamino-[D-Arg^8]-vasopressin (DDAVP); [deamino-[D-3-(3'-pyridyl-Ala)]-[Arg^8]-vasopressin; [1-(beta-Mercapto-beta, beta-cyclopentamethylene propionic acid), 2-(O-methyl)tyrosine]-[Arg^8]-vasopressin; vasopressin metabolite neuropeptide [pGlu^4, Cys^6]; vasopressin metabolite neuropeptide [pGlu^4, Cys^6]; [Lys^8]-deamino vasopressin desglycinamide; [Lys^8]-vasopressin; [Mpr^1,Val^4,DArg^8]-vasopressin; [Phe^2, Ile^3, Orn^8]-vasopressin ([Phe^2, Orn^8]-vasotocin); [Arg^8]-vasotocin; and [d(CH2)5, Tyr(Me)^2, Orn^8]-vasotocin.

Virus related peptides including, but not limited to, fluorogenic human CMV protease substrate; HCV core protein 59-68; HCV NS4A protein 18-40 (JT strain); HCV NS4A protein 21-34 (JT strain); hepatitis B virus receptor binding fragment; hepatitus B virus pre-S region 120-145; [Ala^{127}]-hepatitus B virus pre-S region 120-131; herpes virus inhibitor 2; HIV envelope protein fragment 254-274; HIV gag fragment 129-135; HIV substrate; P 18 peptide; peptide T; [3,5 diiodo-Tyr^7] peptide T; R15K HIV-1 inhibitory peptide; T20; T21; V3 decapeptide P 18-110; and virus replication inhibiting peptide.

While certain analogs, fragments, and/or analog fragments of the various polypeptides have been described above, it is to be understood that other analogs, fragments, and/or analog fragments that retain all or some of the activity of the particular polypeptide may also be useful in embodiments of the present invention. Analogs may be obtained by various means, as will be understood by those skilled in the art. For example, certain amino acids may be substituted for other amino acids in a polypeptide without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. As the interactive capacity and nature of a polypeptide drug defines its biological functional activity, certain amino acid sequence

substitutions can be made in the amino acid sequence and nevertheless remain a polypeptide with like properties.

In making such substitutions, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a polypeptide is generally understood in the art. It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant polypeptide, which in turn defines the interaction of the polypeptide with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics as follows: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5). As will be understood by those skilled in the art, certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a polypeptide with similar biological activity, *i.e.,* still obtain a biological functionally equivalent polypeptide. In making such changes, the substitution of amino acids whose hydropathic indices are within ±2 of each other is preferred, those which are within ±1 of each other are particularly preferred, and those within ±0.5 of each other are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Patent 4,554,101 provides that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein. As detailed in U.S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (±3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ± 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). As is understood by those skilled in the art, an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent polypeptide. In such changes, the substitution of amino acids whose hydrophilicity values are within ±2 of each other is preferred, those which are within ±1 of each other are particularly preferred, and those within ±0.5 of each other are even more

particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions (i.e., amino acids that may be interchanged without significantly altering the biological activity of the polypeptide) that take various of the foregoing characteristics into consideration are well known to those of skill in the art and include, for example: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

As used herein, the term "drug polypeptide" means a polypeptide possessing at least some of the biological activity of drug (e.g., ability to affect the body through drug's primary mechanism of action). For example, an insulin polypeptide may be a polypeptide such as insulin having an A-chain polypeptide and a B-chain polypeptide coupled to the A-chain polypeptide by disulfide bonds. In various embodiments of the present invention, the drug polypeptide preferably possesses a majority of the biological activity of drug, more preferably possesses substantially all of the biological activity of drug, and most preferably possesses all of the biological activity of drug.

As used herein, the term "polypeptide drug analog" means a polypeptide drug wherein one or more of the amino acids have been replaced while retaining some or all of the activity of the drug. The analog is described by noting the replacement amino acids with the position of the replacement as a superscript followed by a description of the drug. For example, "Pro^{B29} insulin, human" means that the lysine typically found at the B29 position of a human insulin molecule has been replaced with proline. As another example, "Pro^{2} calcitonin, human" means that the glycine typically found at the 2 position of a human calcitonin molecule has been replaced with proline. Polypeptide drug analogs may be obtained by various means as described above.

As used herein, the term "polypeptide drug fragment" means a segment of the amino acid sequence found in the polypeptide drug that retains some or all of the activity of the polypeptide drug. Polypeptide drug fragments are denoted by stating the position(s) in an amino acid sequence followed by a description of the amino acid. For example, a "B25-B30 human insulin" fragment would be the six amino acid sequence corresponding to the B25, B26, B27, B28, B29 and B30 positions in the human insulin amino acid sequence.

As used herein, the term "polypeptide drug fragment analog" means a segment of the amino acid sequence found in the polypeptide drug molecule wherein one or more of the

amino acids in the segment have been replaced while retaining some or all of the activity of the polypeptide drug.

As used herein, the term "calcitonin" means calcitonin of one of the following species: chicken, eel, human, porcine, rat, salmon, or the like provided by natural, synthetic, or genetically engineered sources.

As used herein, the term "insulin" means the insulin of one of the following species: human, cow, pig, sheep, horse, dog, chicken, duck, whale, or the like provided by natural, synthetic, or genetically engineered sources. In various embodiments of the present invention, insulin is preferably human insulin.

As used herein, the term "polypeptide" means a peptide having two or more amino acid residues.

As used herein, the term "amphiphilically balanced" means capable of substantially dissolving in water and capable of penetrating biological membranes.

As used herein, the term "polyalkylene glycol" refers to straight or branched polyalkylene glycol polymers such as polyethylene glycol, polypropylene glycol, and polybutylene glycol, and includes the monoalkylether of the polyalkylene glycol. The term "polyalkylene glycol subunit" refers to a single polyalkylene glycol unit. For example, a polyethylene glycol subunit would be $-O-CH_2-CH_2-O-$.

As used herein, the term "lipophilic" means the ability to dissolve in lipids and/or the ability to penetrate, interact with and/or traverse biological membranes, and the term, "lipophilic moiety" or "lipophile" means a moiety which is lipophilic and/or which, when attached to another chemical entity, increases the lipophilicity of such chemical entity. Examples of lipophilic moieties include, but are not limited to, alkyls, fatty acids, esters of fatty acids, cholesteryl, adamantyl and the like.

As used herein, the term "lower alkyl" refers to substituted or unsubstituted alkyl moieties having from one to five carbon atoms.

As used herein, the term "higher alkyl" refers to substituted or unsubstituted alkyl moieties having six or more carbon atoms.

Unless otherwise noted herein, the term "bile salt" includes bile salts and the free acids thereof.

Unless otherwise noted herein, the term "fatty acid" includes fatty acids and pharmaceutically acceptable salts or esters thereof.

As used herein, the term "bile salt component" means a mixture of one or more salts.

As used herein, the term "fatty acid component" means a mixture of one or more fatty acids.

As used herein, the "precipitation point" of a compound or component of the pharmaceutical composition is the pH at which at least 25% of the compound or component precipitates out of the composition. Accordingly, lowering the precipitation point means lowering the pH at which at least 25% of the compound or component precipitates out of the composition. Conversely, raising the precipitation point means raising the pH at which at least 25% of the compound or component precipitates out of the composition.

As used herein, the "solubility point" of a compound or component of the pharmaceutical composition is the pH at which at least 75% of the compound or component is solubilized in the composition. Accordingly, lowering the solubility point means lowering the pH at which at least 75% of the compound or component is solubilized in the composition. Conversely, raising the solubility point means raising the pH at which at least 75% of the compound or component is solubilized in the composition.

As used herein, the term "medium-chain fatty acid" means a saturated or unsaturated fatty acid having from 8 to 14 carbon atoms.

As used herein, the term "long-chain fatty acid" means a saturated or unsaturated fatty acid having greater than 14 carbon atoms.

According to embodiments of the present invention, a pharmaceutical composition comprises a drug-oligomer conjugate, a fatty acid component, and a bile salt component. The drug-oligomer conjugate includes a drug covalently coupled to an oligomeric moiety. The fatty acid component includes a fatty acid, and the bile salt component includes a bile salt.

According to these embodiments of the present invention, the fatty acid component and the bile salt component are present in a weight-to-weight ratio of between 1:5 and 5:1. The fatty acid component and the bile salt component are preferably present in a weight-to-weight ratio of between 1:3 and 3:1, and are more preferably present in a weight-to-weight ratio of 1:2 and 2:1.

According to some embodiments of the present invention, the fatty acid component is present in an amount sufficient to lower the precipitation point of the bile salt compared to a precipitation point of the bile salt if the fatty acid component were not present in the pharmaceutical composition. The fatty acid component is preferably present in an amount sufficient to lower the precipitation point of the bile salt by at least 0.5 pH units, and is more preferably present in an amount sufficient to lower the precipitation point of the bile salt by at least 1.0 pH units.

According to other embodiments of the present invention, the bile salt component is present in an amount sufficient to lower the solubility point of the fatty acid compared to a solubility point of the fatty acid if the bile salt were not present in the pharmaceutical composition. The bile salt component is preferably present in an amount sufficient to lower the solubility point of the fatty acid by at least 0.25 pH units, and is more preferably present in an amount sufficient to lower the solubility point of the fatty acid by at least 0.5 pH units.

According to still other embodiments of the present invention, the fatty acid component is present in an amount sufficient to lower the precipitation point of the bile salt compared to a precipitation point of the bile salt if the fatty acid were not present in the pharmaceutical composition as described above, and the bile salt component is present in an amount sufficient to lower the solubility point of the fatty acid compared to a solubility point of the fatty acid if the bile salt were not present in the pharmaceutical composition as described above.

The bile salt in the bile salt component may be various bile salts as will be understood by those skilled in the art including unconjugated and conjugated bile salts. Unconjugated bile salts are bile salts in which the primary side chain has a single carboxyl group which is at the terminal position and which is unsubstituted. Exemplary unconjugated bile salts include, but are not limited to, cholate, ursodeoxycholate, chenodeoxycholate, and deoxycholate. Conjugated bile salts are bile salts in which the primary side chain has a carboxyl group which is substituted with, for example, an amino acid derivative linked via its nitrogen atom to the carboxyl group. Exemplary conjugated bile salts include, but are not limited to, taurocholate, glycocholate, taurodeoxycholate, and glycodeoxycholate. Mixtures of the various unconjugated and/or conjugated bile salts may also be used. The bile salt is preferably a pharmaceutically acceptable salt of cholic acid. More preferably, the bile salt is sodium cholate. Still more preferably, the bile salt component consists essentially of sodium cholate.

The fatty acid in the fatty acid component may be various fatty acids as will be understood by those skilled in the art including natural and synthetic fatty acids. The fatty acid preferably has between a lower limit of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 carbon atoms and an upper limit of 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22 carbon atoms. The fatty acid may be either saturated or unsaturated. Exemplary saturated fatty acids include, but are not limited to, ethanoic acid, propanoic acid, butanoic acid, pentanoic acid, hexanoic acid, heptanoic acid, octanoic acid, nonanoic acid, decanoic acid, undecanoic acid, dodecanoic acid, tridecanoic acid, tetradecanoic acid,

pentadecanoic acid, hexadecanoic acid, heptadecanoic acid, octadecanoic acid, nonadecanoic acid, eicosanoic acid, one or more of which may be referred to by their common names such as acetic acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid, behenic acid, lignoceric acid, and cerotic acid. Exemplary unsaturated fatty acids include, but are not limited to, cis-9-octadecenoic acid, trans-9-octadecenoic acid, 9,12-octadecatrienoic acid, 9,12,15-octadecenoic acid, and 5,8,11,14-eicosatetraenoic acid, one or more of which may be referred to by their common names such as oleic acid, elaidic acid, linoleic acid, linolenic acid, and arachidonic acid.

In some embodiments, the fatty acid component comprises a mixture of two or more fatty acids. In other embodiments, the fatty acid component comprises a medium-chain fatty acid and a long-chain fatty acid. The medium-chain fatty acid is preferably capric acid, lauric acid, or a mixture thereof. The long-chain fatty acid is preferably oleic acid.

The drug may be various drugs as described above that can be conjugated to oligomers as will be understood by those skilled in the art. In some embodiments, the drug is a small molecule drug such as the anti-cancer drug taxane, which is described in U.S. Patent No. 6,380,405 to Ekwuribe et al., the disclosure of which is incorporated herein in its entirety. In other embodiments, the drug is a polypeptide drug, such as those described above. The polypeptide drug is preferably an insulin polypeptide, a calcitonin polypeptide, a growth hormone polypeptide, or an etoposide polypeptide.

The oligomer may be various oligomers as will be understood by those skilled in the art. In general, the oligomer may be any oligomer capable of being coupled to a drug as will be understood by those skilled in the art. For example, the oligomer may be a poly-dispersed oligomer as described in U.S. Patent No. 4,179,337 to Davis et al.; U.S. Patent No. 5,567,422 to Greenwald; U.S. Patent No. 5,359,030 to Ekwuribe; U.S. Patent No. 5,438,040 to Ekwuribe, U.S. Patent No. 5,681,811 to Ekwuribe, U.S. Patent No. 6,309,633 to Ekwuribe et al., and U.S. Patent No. 6,380,405 to Ekwuribe et al., the disclosures of each of which are incorporated herein by reference in their entireties. As another example, the oligomer may be a non-polydispersed oligomer as described in U.S. Patent Application Serial No. 09/873,731 filed June 4, 2001 by Ekwuribe et al. entitled "Methods of Synthesizing Substantially Monodispersed Mixtures of Polymers Having Polyethylene Glycol Mixtures"; U.S. Patent Application Serial No. 09/873,797 filed June 4, 2001 by Ekwuribe et al. entitled "Mixtures of Drug-Oligomer Conjugates Comprising Polyalkylene Glycol, Uses Thereof, and Methods of Making Same"; U.S. Patent Application Serial No. 09/873,899 filed June 4, 2001 by Ekwuribe et al. entitled "Mixtures of Insulin Drug-Oligomer Conjugates Comprising

Polyalkylene Glycol, Uses Thereof, and Methods of Making Same"; U.S. Patent Application Serial No. 09/873,777 filed June 4, 2001 by Ekwuribe et al. entitled "Mixtures of Calcitonin Drug-Oligomer Conjugates Comprising Polyalkylene Glycol, Uses Thereof, and Methods of Making Same"; and U.S. Patent Application Serial No. 09/873,757 filed June 4, 2001 by Ekwuribe et al. entitled "Mixtures of Growth Hormone Drug-Oligomer Conjugates Comprising Polyalkylene Glycol, Uses Thereof, and Methods of Making Same," the disclosures of each of which are incorporated herein in their entireties.

In some embodiments, the oligomer comprises a hydrophilic moiety as will be understood by those skilled in the art including, but not limited to, polyalkylene glycols such as polyethylene glycol or polypropylene glycol, polyoxyethylenated polyols, copolymers thereof and block copolymers thereof, provided that the hydrophilicity of the block copolymers is maintained. The hydrophilic moiety is preferably a polyalkylene glycol moiety. The polyalkylene glycol moiety has at least 1, 2, 3, 4, 5, 6 or 7 polyalkylene glycol subunits. The polyalkylene glycol moiety preferably has between a lower limit of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, or 49 polyalkylene glycol subunits and an upper limit of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more polyalkylene glycol subunits. The polyalkylene glycol moiety more preferably has between a lower limit of 2, 3, 4, 5, or 6 polyalkylene glycol subunits and an upper limit of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 polyalkylene glycol subunits. Even more preferably, the polyalkylene glycol moiety has between a lower limit of 3, 4, 5, or 6 polyalkylene glycol subunits and an upper limit of 5, 6, 7, 8, 9, 10, 11, or 12 polyalkylene glycol subunits. The polyalkylene glycol moiety still more preferably has between a lower limit of 4, 5, or 6 polyalkylene glycol subunits and an upper limit of 6, 7, or 8 polyalkylene glycol subunits. The polyalkylene glycol moiety of the oligomer is preferably a lower alkyl polyalkylene glycol moiety such as a polyethylene glycol moiety, a polypropylene glycol moiety, or a polybutylene glycol moiety. When the polyalkylene glycol moiety is a polypropylene glycol moiety, the moiety preferably has a uniform (i.e., not random) structure. An exemplary polypropylene glycol moiety having a uniform structure is as follows:

$$-O-\underset{CH_3}{\underset{|}{CH}}-CH_2-O-\underset{CH_3}{\underset{|}{CH}}-CH_2-O-\underset{CH_3}{\underset{|}{CH}}-CH_2-O-\underset{CH_3}{\underset{|}{CH}}-CH_2-O-$$

This uniform polypropylene glycol structure may be described as having only one methyl substituted carbon atom adjacent each oxygen atom in the polypropylene glycol chain. Such uniform polypropylene glycol moieties may exhibit both lipophilic and hydrophilic characteristics.

The oligomer may comprise one or more other moieties as will be understood by those skilled in the art including, but not limited to, additional hydrophilic moieties, lipophilic moieties, spacer moieties, linker moieties, and terminating moieties. The various moieties in the oligomer are covalently coupled to one another by either hydrolyzable or non-hydrolyzable bonds.

The oligomer may further comprise one or more additional hydrophilic moieties (i.e., moieties in addition to the polyalkylene glycol moiety) including, but not limited to, sugars, polyalkylene glycols, and polyamine/PEG copolymers. Adjacent polyalkylene glycol moieties will be considered to be the same moiety if they are coupled by ether bonds. For example, the moiety

$$-O-C_2H_4-O-C_2H_4-O-C_2H_4-O-C_2H_4-O-C_2H_4-O-C_2H_4-$$

is a single polyethylene glycol moiety having six polyethylene glycol subunits. If this moiety were the only hydrophilic moiety in the oligomer, the oligomer would not contain an additional hydrophilic moiety. Adjacent polyethylene glycol moieties will be considered to be different moieties if they are coupled by a bond other than an ether bond. For example, the moiety

$$-O-C_2H_4-O-C_2H_4-O-C_2H_4-O-C_2H_4-\overset{\displaystyle O}{\overset{\|}{C}}-O-C_2H_4-O-C_2H_4-$$

is a polyethylene glycol moiety having four polyethylene glycol subunits and an additional hydrophilic moiety having two polyethylene glycol subunits. Preferably, oligomers according to embodiments of the present invention comprise a polyalkylene glycol moiety and no additional hydrophilic moieties.

The oligomer preferably further comprises one or more lipophilic moieties as will be understood by those skilled in the art. The lipophilic moiety has at least 1, 2, 3, 4, 5, or 6 carbon atoms. The lipophilic moiety preferably has between a lower limit of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29 carbon atoms and an upper limit of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 carbon atoms. The lipophilic moiety more preferably has between a lower limit of 2, 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms and an upper limit of 4, 5, 6,

7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22 carbon atoms. The lipophilic moiety even more preferably has between a lower limit of 3, 4, 5, 6, 7, 8, or 9 carbon atoms and an upper limit of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 carbon atoms. The lipophilic moiety still more preferably has between a lower limit of 3, 4, 5, 6, or 7 carbon atoms and an upper limit of 6, 7, 8, 9, or 10 carbon atoms. The lipophilic moiety is preferably selected from the group consisting of saturated or unsaturated, linear or branched alkyl moieties, saturated or unsaturated, linear or branched fatty acid moieties, cholesterol, and adamantane. Exemplary alkyl moieties include, but are not limited to, saturated, linear alkyl moieties such as methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, octadecyl, nonadecyl and eicosyl; saturated, branched alkyl moieties such as isopropyl, *sec*-butyl, *tert*-butyl, 2-methylbutyl, *tert*-pentyl, 2-methyl-pentyl, 3-methylpentyl, 2-ethylhexyl, 2-propylpentyl; and unsaturated alkyl moieties derived from the above saturated alkyl moieties including, but not limited to, vinyl, allyl, 1-butenyl, 2-butenyl, ethynyl, 1-propynyl, and 2-propynyl. Exemplary fatty acid moieties include, but are not limited to, unsaturated fatty acid moieties such as lauroleate, myristoleate, palmitoleate, oleate, elaidate, erucate, linoleate, linolenate, arachidonate, eicosapentaentoate, and docosahexaenoate; and saturated fatty acid moieties such as acetate, caproate, caprylate, caprate, laurate, arachidate, behenate, lignocerate, and cerotate. The fatty acid moiety can be natural or synthetic.

The oligomer may further comprise one or more spacer moieties as will be understood by those skilled in the art. Spacer moieties may, for example, be used to separate a hydrophilic moiety from a lipophilic moiety, to separate a lipophilic moiety or hydrophilic moiety from the drug, to separate a first hydrophilic or lipophilic moiety from a second hydrophilic or lipophilic moiety, or to separate a hydrophilic moiety or lipophilic moiety from a linker moiety. Spacer moieties are preferably selected from the group consisting of sugar, cholesterol and glycerine moieties. Sugar moieties may be various sugar moieties as will be understood by those skilled in the art including, but not limited to, monosaccharide moieties and disaccharide moieties. Preferred monosaccharide moieties have between 4 and 6 carbon atoms.

The oligomer may further comprise one or more linker moieties that are used to couple the oligomer with the drug as will be understood by those skilled in the art. Linker moieties are preferably selected from the group consisting of alkyl and fatty acid moieties. The alkyl linker moiety may be a saturated or unsaturated, linear or branched alkyl moiety as will be understood by those skilled in the art including, but not limited to, methyl, ethyl,

propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, octadecyl, nonadecyl, eicosyl, isopropyl, *sec*-butyl, *tert*-butyl, 2-methylbutyl, *tert*-pentyl, 2-methyl-pentyl, 3-methylpentyl, 2-ethylhexyl, 2-propylpentyl, vinyl, allyl, 1-butenyl, 2-butenyl, ethynyl, 1-propynyl, and 2-propynyl. The alkoxy moiety may be various alkoxy moieties including, but not limited to, methoxy, ethoxy, propoxy, butoxy, pentyloxy, hexyloxy, heptyloxy, octyloxy, nonyloxy, decyloxy, undecyloxy, dodecyloxy, tridecyloxy, tetradecyloxy, pentadecyloxy, hexadecyloxy, octadecyloxy, nonadecyloxy, eicosyloxy, isopropoxy, *sec*-butoxy, *tert*-butoxy, 2-methylbutoxy, *tert*-pentyloxy, 2-methyl-pentyloxy, 3-methylpentyloxy, 2-ethylhexyloxy, 2-propylpentyloxy, vinyloxy, allyloxy, 1-butenyloxy, 2-butenyloxy, ethynyloxy, 1-propynyloxy, and 2-propynyloxy. The alkyl linker moiety may have between a lower limit of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms and an upper limit of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 carbon atoms, and preferably has between 1, 2, 3, 4, or 5 carbon atoms and 8, 9, 10, 11, or 12 carbon atoms. The fatty acid linker moiety may be a saturated or unsaturated, linear or branched fatty acid moiety as will be understood by those skilled in the art including, but not limited to, lauroleate, myristoleate, palmitoleate, oleate, elaidate, erucate, linoleate, linolenate, arachidonate, eicosapentaentoate, docosahexaenoate, acetate, caproate, caprylate, caprate, laurate, arachidate, behenate, lignocerate, and cerotate. The fatty acid linker moiety may be a natural or synthetic fatty acid. The fatty acid linker moiety may have between a lower limit of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms and an upper limit of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 carbon atoms and preferably has between 1, 2, 3, 4, or 5 carbon atoms and 8, 10, 12, 14 or 16 carbon atoms. When the linker moiety is a fatty acid, the oligomeric moiety is preferably coupled to the drug via the carbonyl group of a carboxylic acid moiety of the fatty acid.

The oligomer may further comprise one or more terminating moieties at the one or more ends of the oligomer, which are not coupled to the drug. The terminating moiety is preferably an alkyl or alkoxy moiety. The alkyl or alkoxy moiety preferably has between a lower limit of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms and an upper limit of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 carbon atoms. The alkyl or alkoxy moiety more preferably has between a lower limit of 1, 2, 3, 4, 5, 6, or 7 carbon atoms and an upper limit of 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 carbon atoms. The alkyl or alkoxy moiety even more preferably has

between a lower limit of 1, 2, 3, 4, or 5 carbon atoms and an upper limit of 5, 6, 7, 8, 9, or 10 carbon atoms. The alkyl or alkoxy moiety still more preferably has between a lower limit of 1, 2, 3, or 4 carbon atoms and an upper limit of 5, 6, or 7 carbon atoms. The alkyl moiety may be a linear or branched, saturated or unsaturated alkyl moiety as will be understood by those skilled in the art including, but not limited to, methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, octadecyl, nonadecyl, eicosyl, isopropyl, *sec*-butyl, *tert*-butyl, 2-methylbutyl, *tert*-pentyl, 2-methyl-pentyl, 3-methylpentyl, 2-ethylhexyl, 2-propylpentyl, vinyl, allyl, 1-butenyl, 2-butenyl, ethynyl, 1-propynyl, and 2-propynyl. The alkoxy moiety may be various alkoxy moieties including, but not limited to, methoxy, ethoxy, propoxy, butoxy, pentyloxy, hexyloxy, heptyloxy, octyloxy, nonyloxy, decyloxy, undecyloxy, dodecyloxy, tridecyloxy, tetradecyloxy, pentadecyloxy, hexadecyloxy, octadecyloxy, nonadecyloxy, eicosyloxy, isopropoxy, *sec*-butoxy, *tert*-butoxy, 2-methylbutoxy, *tert*-pentyloxy, 2-methyl-pentyloxy, 3-methylpentyloxy, 2-ethylhexyloxy, 2-propylpentyloxy, vinyloxy, allyloxy, 1-butenyloxy, 2-butenyloxy, ethynyloxy, 1-propynyloxy, and 2-propynyloxy. The terminating moiety is more preferably a lower alkyl moiety such as methyl, ethyl, propyl, isopropyl, butyl, *sec*-butyl, *tert*-butyl, pentyl, or *tert*-pentyl, or a lower alkoxy moiety such as methoxy, ethoxy, propoxy, isopropoxy, butoxy, *sec*-butoxy, *tert*-butoxy, pentyloxy, or *tert*-pentyloxy. Most preferably, the terminating moiety is methyl or methoxy. While the terminating moiety is preferably an alkyl or alkoxy moiety, it is to be understood that the terminating moiety may be various moieties as will be understood by those skilled in the art including, but not limited to, sugars, cholesterol, alcohols, and fatty acids.

According to embodiments of the present invention, the drug-oligomer conjugate comprises the structure of Formula I:

$$\text{Drug}—\text{B}—\text{L}_j—\text{G}_k—\text{R}—\text{G}'_m—\text{R}'—\text{G}''_n—\text{T} \qquad \text{(I)}$$

wherein:

Drug is a drug similar to those described above;

B is a bonding moiety;

L is a linker moiety;

G, G' and G" are individually selected spacer moieties;

R is a lipophilic moiety and R' is a polyalkylene glycol moiety, or R' is the lipophilic moiety and R is the polyalkylene glycol moiety;

T is a terminating moiety; and

j, k, m and n are individually 0 or 1.

The bonding moiety is preferably selected from the group consisting of an ester moity, a thio-ester moiety, an ether moiety, a carbamate moiety, a thio-carbamate moiety, a carbonate moiety, a thio-carbonate moiety, an amide moiety, a urea moiety, and a covalent bond. The linker moiety, spacer moieties, lipophilic moiety, polyalkylene glycol moiety, and terminating moiety are similar to those described above. Preferably, oligomers of these embodiments do not include spacer moieties (i.e., k, m and n are preferably 0).

In some embodiments of conjugate according to Formula I, the drug is a polypeptide drug as described above. The polypeptide drug is preferably an insulin polypeptide, a calcitonin polypeptide, or a growth hormone polypeptide. The insulin polypeptide is preferably insulin and is more preferably human insulin. The calcitonin polypeptide is preferably salmon calcitonin or human calcitonin, and is more preferably salmon calcitonin. However, it is to be understood that the calcitonin polypeptide may be selected from various calcitonin polypeptides known to those skilled in the art including, for example, calcitonin precursor peptides, calcitonin, calcitonin analogs, calcitonin fragments, and calcitonin fragment analogs. Calcitonin precursor peptides include, but are not limited to, katacalcin (PDN-21) (C-procalcitonin), and N-proCT (amino-terminal procalcitonin cleavage peptide), human. Calcitonin analogs may be provided by substitution of one or more amino acids in calcitonin as described above. Calcitonin fragments include, but are not limited to, calcitonin 1-7, human; and calcitonin 8-32, salmon. Calcitonin fragment analogs may be provided by substitution of one or more of the amino acids in a calcitonin fragment as described above.

In other embodiments, the drug-oligomer conjugate comprises the structure of Formula II:

$$\text{Drug}{-}X(CH_2)_mY(C_2H_4O)_nR \qquad \text{(II)}$$

wherein:

Drug is a drug similar to those described above;

X is -C(O)- or -O-;

Y is an ester, an ether, a carbamate, a carbonate, or an amide bonding moiety, and is preferably an ether bonding moiety;

m is between a lower limit of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 and an upper limit of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30, is more preferably between a lower limit of 2, 3, 4, 5,

6, 7, 8, 9, or 10 and an upper limit of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22, is even more preferably between a lower limit of 3, 4, 5, 6, 7, 8, or 9 and an upper limit of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14, and is still more preferably has between a lower limit of 3, 4, 5, 6, or 7 and an upper limit of 6, 7, 8, 9, or 10;

n is between a lower limit of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 and an upper limit of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50, is more preferably between a lower limit of 2, 3, 4, 5, or 6 and an upper limit of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, is even more preferably between a lower limit of 3, 4, 5, or 6 and an upper limit of 5, 6, 7, 8, 9, 10, 11, or 12 polyalkylene glycol subunits, is still more preferably between a lower limit of 4, 5, or 6 and an upper limit of 6, 7, or 8 polyalkylene glycol subunits, and is most preferably 7; and

R is a terminating moiety similar to those described above.

In some embodiments of conjugates according to Formula II, the drug is a polypeptide drug as described above. The polypeptide drug is preferably an insulin polypeptide, a calcitonin polypeptide, or a growth hormone polypeptide. The insulin polypeptide is preferably insulin and is more preferably human insulin. The calcitonin polypeptide is preferably salmon calcitonin or human calcitonin, and is more preferably salmon calcitonin. However, it is to be understood that the calcitonin polypeptide may be selected from various calcitonin polypeptides known to those skilled in the art including, for example, calcitonin precursor peptides, calcitonin, calcitonin analogs, calcitonin fragments, and calcitonin fragment analogs. Calcitonin precursor peptides include, but are not limited to, katacalcin (PDN-21) (C-procalcitonin), and N-proCT (amino-terminal procalcitonin cleavage peptide), human. Calcitonin analogs may be provided by substitution of one or more amino acids in calcitonin as described above. Calcitonin fragments include, but are not limited to, calcitonin 1-7, human; and calcitonin 8-32, salmon. Calcitonin fragment analogs may be provided by substitution of one or more of the amino acids in a calcitonin fragment as described above.

In still other embodiments, the drug-oligomer conjugate comprises the structure of Formula III:

$$\text{Drug}-X_1-(CH_2)_m(OC_2H_4)_nOR \qquad \text{(III)}$$

wherein:

Drug is a drug similar to those described above;

X_1 is -C(O)- or -O-;

m is between a lower limit of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 and an upper limit of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30, is preferably between a lower limit of 2, 3, 4, 5, 6, 7, 8, 9, or 10 and an upper limit of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22, is more preferably between a lower limit of 3, 4, 5, 6, 7, 8, or 9 and an upper limit of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14, and is still more preferably has between a lower limit of 3, 4, 5, 6, or 7 and an upper limit of 6, 7, 8, 9, or 10;

n is between a lower limit of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 and an upper limit of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50, is preferably between a lower limit of 2, 3, 4, 5, or 6 and an upper limit of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, is more preferably between a lower limit of 3, 4, 5, or 6 and an upper limit of 5, 6, 7, 8, 9, 10, 11, or 12 polyalkylene glycol subunits, and is still more preferably between a lower limit of 4, 5, or 6 and an upper limit of 6, 7, or 8 polyalkylene glycol subunits; and

R is a terminating moiety similar to those described above.

In some embodiments of conjugate according to Formula III, the drug is a polypeptide drug as described above. The polypeptide drug is preferably an insulin polypeptide, a calcitonin polypeptide, or a growth hormone polypeptide. The insulin polypeptide is preferably insulin and is more preferably human insulin. The calcitonin polypeptide is preferably salmon calcitonin or human calcitonin, and is more preferably salmon calcitonin. However, it is to be understood that the calcitonin polypeptide may be selected from various calcitonin polypeptides known to those skilled in the art including, for example, calcitonin precursor peptides, calcitonin, calcitonin analogs, calcitonin fragments, and calcitonin fragment analogs. Calcitonin precursor peptides include, but are not limited to, katacalcin (PDN-21) (C-procalcitonin), and N-proCT (amino-terminal procalcitonin cleavage peptide), human. Calcitonin analogs may be provided by substitution of one or more amino acids in calcitonin as described above. Calcitonin fragments include, but are not limited to, calcitonin 1-7, human; and calcitonin 8-32, salmon. Calcitonin fragment analogs may be provided by substitution of one or more of the amino acids in a calcitonin fragment as described above.

In yet other embodiments, the drug-oligomer conjugate comprises the structure of Formula IV:

$$\text{Drug}—\overset{\overset{\displaystyle O}{\|}}{C}\text{-}(CH_2)_m(OC_2H_4)_nOR \qquad (IV)$$

wherein:

Drug is a drug similar to those described above;

m is between a lower limit of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 and an upper limit of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30, is more preferably between a lower limit of 2, 3, 4, 5, 6, 7, 8, 9, or 10 and an upper limit of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22, is even more preferably between a lower limit of 3, 4, 5, 6, 7, 8, or 9 and an upper limit of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14, and is still more preferably between a lower limit of 3, 4, 5, 6, or 7 and an upper limit of 6, 7, 8, 9, or 10;

n is between a lower limit of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 and an upper limit of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50, is more preferably between a lower limit of 2, 3, 4, 5, or 6 and an upper limit of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, is even more preferably between a lower limit of 3, 4, 5, or 6 and an upper limit of 5, 6, 7, 8, 9, 10, 11, or 12 polyalkylene glycol subunits, and is still more preferably between a lower limit of 4, 5, or 6 and an upper limit of 6, 7, or 8 polyalkylene glycol subunits; and

R is a terminating moiety similar to those described above.

In some embodiments of conjugates according to Formula IV, the drug is a polypeptide drug as described above. The polypeptide drug is preferably an insulin polypeptide, a calcitonin polypeptide, or a growth hormone polypeptide. The insulin polypeptide is preferably insulin and is more preferably human insulin. The calcitonin polypeptide is preferably salmon calcitonin or human calcitonin, and is more preferably salmon calcitonin. However, it is to be understood that the calcitonin polypeptide may be selected from various calcitonin polypeptides known to those skilled in the art including, for example, calcitonin precursor peptides, calcitonin, calcitonin analogs, calcitonin fragments, and calcitonin fragment analogs. Calcitonin precursor peptides include, but are not limited to, katacalcin (PDN-21) (C-procalcitonin), and N-proCT (amino-terminal procalcitonin cleavage peptide), human. Calcitonin analogs may be provided by substitution of one or more amino acids in calcitonin as described above. Calcitonin fragments include, but are not limited to, calcitonin 1-7,

human; and calcitonin 8-32, salmon. Calcitonin fragment analogs may be provided by substitution of one or more of the amino acids in a calcitonin fragment as described above.

When the drug in the drug-oligomer conjugate of Formula IV is salmon calcitonin, the drug-oligomer preferably comprises the following structure:

$$\text{Salmon calcitonin}\left[\overset{\displaystyle O}{\overset{\|}{C}}\text{-}(CH_2)_7(OC_2H_4)_7OCH_3\right]_2$$

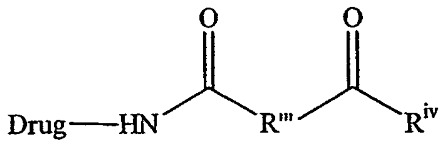
where one of the oligomers is coupled to lysine at position 11 of the salmon calcitonin and where the other oligomer is coupled to lysine at position 18 of the salmon calcitonin. This salmon calcitonin-oligomer conjugate is referred to as CT-025.

In still other embodiments, the drug-oligomer conjugate comprises the structure of Formula V:

$$\text{Drug}-\overset{\displaystyle O}{\overset{\|}{C}}\text{-}(CH_2)_5(OC_2H_4)_7OCH_3 \qquad (V).$$

In some embodiments according to conjugates of Formula V, the drug is a polypeptide drug as described above. The polypeptide drug is preferably an insulin polypeptide, a calcitonin polypeptide, a C-peptide polypeptide, or a growth hormone polypeptide. The insulin polypeptide is preferably insulin and is more preferably human insulin. When the drug is human insulin and the conjugate of Formula V consists of the single oligomer coupled to the Lysine at the B29 position of the human insulin, the insulin-oligomer conjugate is referred to as HIM2. The C-peptide polypeptide is preferably human C-peptide, having glutamate at its N-terminus and lysine at its C-terminus, with the oligomer $CH_3O(C_2H_4O)C(O)$- attached to the ε-amino group of the lysine of the human C-peptide. The calcitonin polypeptide is preferably salmon calcitonin or human calcitonin, and is more preferably salmon calcitonin. However, it is to be understood that the calcitonin polypeptide may be selected from various calcitonin polypeptides known to those skilled in the art including, for example, calcitonin precursor peptides, calcitonin, calcitonin analogs, calcitonin fragments, and calcitonin fragment analogs. Calcitonin precursor peptides include, but are not limited to, katacalcin (PDN-21) (C-procalcitonin), and N-proCT (amino-terminal procalcitonin cleavage peptide), human. Calcitonin analogs may be provided by substitution of one or more amino acids in calcitonin as described above. Calcitonin fragments include, but are not limited to, calcitonin 1-7, human; and calcitonin 8-32, salmon. Calcitonin fragment analogs may be provided by substitution of one or more of the amino acids in a calcitonin fragment as described above.

In still other embodiments of the present invention, the drug-oligomer conjugate comprises the structure of Formula VI:



(VI)

wherein:

Drug is a drug similar to those described above;

R''' is a hydrophilic moiety; preferably a polyalkylene glycol moiety; more preferably a lower polyalkylene glycol moiety; and still more preferably a polyethylene glycol moiety or polypropylene glycol moiety, where the polyalkylene glycol moiety has at least 1, 2, or 3 polyalkylene glycol subunits; and R^{iv} is a lipophilic moiety; preferably an alkyl moiety having between 1 and 24 carbon atoms; more preferably a lower alkyl moiety; or

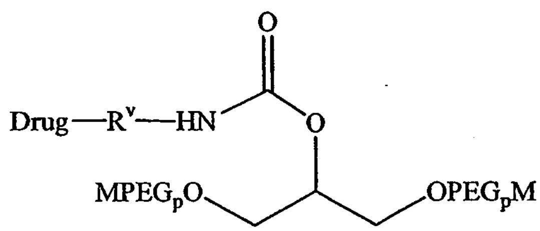
R^{iv} is a hydrophilic moiety; preferably a polyalkylene glycol moiety; more preferably a lower polyalkylene glycol moiety; and still more preferably a polyethylene glycol moiety or polypropylene glycol moiety, where the polyalkylene glycol moiety has at least 1, 2, or 3 polyalkylene glycol subunits; and R''' is a lipophilic moiety; preferably an alkyl moiety having between 1 and 24 carbon atoms; more preferably a lower alkyl moiety.

In some embodiments of conjugate according to Formula VI, the drug is a polypeptide drug as described above. The polypeptide drug is preferably an insulin polypeptide, a calcitonin polypeptide, or a growth hormone polypeptide. The insulin polypeptide is preferably insulin and is more preferably human insulin. The calcitonin polypeptide is preferably salmon calcitonin or human calcitonin, and is more preferably salmon calcitonin. However, it is to be understood that the calcitonin polypeptide may be selected from various calcitonin polypeptides known to those skilled in the art including, for example, calcitonin precursor peptides, calcitonin, calcitonin analogs, calcitonin fragments, and calcitonin fragment analogs. Calcitonin precursor peptides include, but are not limited to, katacalcin (PDN-21) (C-procalcitonin), and N-proCT (amino-terminal procalcitonin cleavage peptide), human. Calcitonin analogs may be provided by substitution of one or more amino acids in calcitonin as described above. Calcitonin fragments include, but are not limited to, calcitonin 1-7, human; and calcitonin 8-32, salmon. Calcitonin fragment analogs may be provided by substitution of one or more of the amino acids in a calcitonin fragment as described above.

When the drug portion of the drug-oligomer conjugate of Formula VI is human insulin, R''' is preferably polyethylene glycol having between a lower limit of 1, 2, 3, 4, 5, 6,

or 7 polyethylene glycol subunits and an upper limit of 2, 3, 4, 5, 6, 7, 8, 9, or 10 polyethylene glycol subunits or R''' is polypropylene glycol having between a lower limit of 1, 2, 3, 4, 5, 6, or 7 polypropylene glycol subunits and an upper limit of 2, 3, 4, 5, 6, 7, 8, 9, or 10 polypropylene glycol subunits, and R^{iv} is preferably alkyl having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms. More preferably, R''' is polyethylene glycol having between a lower limit of 1, 2, 3, or 4 polyethylene glycol subunits and an upper limit of 2, 3, 4, 5, 6, or 7 polyethylene glycol subunits or R''' is polypropylene glycol having between a lower limit of 1, 2, 3, or 4 polypropylene glycol subunits and an upper limit of 2, 3, 4, 5, 6, or 7 polypropylene glycol subunits, and R^{iv} is alkyl having 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms. Still more preferably, R''' is polyethylene glycol having between a lower limit of 1, 2, or 3 polyethylene glycol subunits and an upper limit of 3, 4, or 5 polyethylene glycol subunits or R''' is polypropylene glycol having between a lower limit of 1, 2, or 3 polypropylene glycol subunits and an upper limit of 3, 4, or 5 polypropylene glycol subunits, and R^{iv} is alkyl having 3, 4, 5, or 6 carbon atoms.

In yet other embodiments of the present invention, the drug-oligomer conjugate comprises the structure of Formula VII:



(VII)

wherein:

Drug is a drug similar to those described above;

R^v is an alkyl or a fatty acid moiety as described above with reference to the lipophilic moiety; and

p is between a lower limit of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 and an upper limit of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30, is more preferably between a lower limit of 2, 3, 4, 5, 6, 7, 8, 9, or 10 and an upper limit of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22, is even more preferably between a lower limit of 3, 4, 5, 6, 7, 8, or 9 and an upper limit of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14, and is still more preferably between a lower limit of 3, 4, 5, 6, or 7 and an upper limit of 6, 7, 8, 9, or 10.

In some embodiments of conjugate according to Formula VII, the drug is a polypeptide drug as described above. The polypeptide drug is preferably an insulin polypeptide, a calcitonin polypeptide, or a growth hormone polypeptide. The insulin polypeptide is preferably insulin and is more preferably human insulin. The calcitonin polypeptide is preferably salmon calcitonin or human calcitonin, and is more preferably salmon calcitonin. However, it is to be understood that the calcitonin polypeptide may be selected from various calcitonin polypeptides known to those skilled in the art including, for example, calcitonin precursor peptides, calcitonin, calcitonin analogs, calcitonin fragments, and calcitonin fragment analogs. Calcitonin precursor peptides include, but are not limited to, katacalcin (PDN-21) (C-procalcitonin), and N-proCT (amino-terminal procalcitonin cleavage peptide), human. Calcitonin analogs may be provided by substitution of one or more amino acids in calcitonin as described above. Calcitonin fragments include, but are not limited to, calcitonin 1-7, human; and calcitonin 8-32, salmon. Calcitonin fragment analogs may be provided by substitution of one or more of the amino acids in a calcitonin fragment as described above.

In the various embodiments described above, the oligomer is covalently coupled to the drug. In some embodiments, the oligomer is coupled to the drug utilizing a hydrolyzable bond (e.g., an ester or carbonate bond). A hydrolyzable coupling may provide a drug-oligomer conjugate that acts as a prodrug. In certain instances, for example where the drug-oligomer conjugate is biologically inactive (i.e., the conjugate lacks the ability to affect the body through the insulin polypeptide's primary mechanism of action), a hydrolyzable coupling may provide for a time-release or controlled-release effect, providing the biologically active drug over a given time period as one or more oligomers are cleaved from their respective biologically inactive drug-oligomer conjugates to provide the biologically active drug. In other embodiments, the oligomer is coupled to the drug utilizing a non-hydrolyzable bond (e.g., a carbamate, amide, or ether bond). Use of a non-hydrolyzable bond may be preferable when it is desirable to allow the biologically inactive drug-oligomer conjugate to circulate in the bloodstream for an extended period of time, preferably at least 2 hours.

Oligomers employed in the various embodiments described above are commercially available or may be synthesized by various methods as will be understood by those skilled in the art. For example, polydispersed oligomers may be synthesized by the methods provided in one or more of the following references: U.S. Patent No. 4,179,337 to Davis et al.; U.S. Patent No. 5,567,422 to Greenwald; U.S. Patent No. 5,359,030 to Ekwuribe; U.S. Patent No. 5,438,040 to Ekwuribe, U.S. Patent No. 5,681,811 to Ekwuribe, U.S. Patent No. 6,309,633 to

Ekwuribe et al. Non-polydispersed (e.g., substantially monodispersed and monodispersed) oligomers may be synthesized by methods provided in one or more of the following references: U.S. Patent Application Serial No. 09/873,731 filed June 4, 2001 by Ekwuribe et al. entitled "Methods of Synthesizing Substantially Monodispersed Mixtures of Polymers Having Polyethylene Glycol Mixtures"; U.S. Patent Application Serial No. 09/873,797 filed June 4, 2001 by Ekwuribe et al. entitled "Mixtures of Drug-Oligomer Conjugates Comprising Polyalkylene Glycol, Uses Thereof, and Methods of Making Same"; U.S. Patent Application Serial No. 09/873,899 filed June 4, 2001 by Ekwuribe et al. entitled "Mixtures of Insulin Drug-Oligomer Conjugates Comprising Polyalkylene Glycol, Uses Thereof, and Methods of Making Same"; U.S. Patent Application Serial No. 09/873,777 filed June 4, 2001 by Ekwuribe et al. entitled "Mixtures of Calcitonin Drug-Oligomer Conjugates Comprising Polyalkylene Glycol, Uses Thereof, and Methods of Making Same"; and U.S. Patent Application Serial No. 09/873,757 filed June 4, 2001 by Ekwuribe et al. entitled "Mixtures of Growth Hormone Drug-Oligomer Conjugates Comprising Polyalkylene Glycol, Uses Thereof, and Methods of Making Same." Oligomers according to embodiments of the present invention are preferably substantially monodispersed and are more preferably monodispersed. Exemplary methods for synthesizing various monodispersed oligomers are provided in Examples 1 through 10 below.

In the various embodiments described above, more than one oligomer (i.e., a plurality of oligomers) may be coupled to the drug. The oligomers in the plurality are preferably the same. However, it is to be understood that the oligomers in the plurality may be different from one another, or, alternatively, some of the oligomers in the plurality may be the same and some may be different. When a plurality of oligomers are coupled to the drug, it may be preferable to couple one or more of the oligomers to the drug with hydrolyzable bonds and couple one or more of the oligomers to the drug with non-hydrolyzable bonds. Alternatively, all of the bonds coupling the plurality of oligomers to the drug may be hydrolyzable, but have varying degrees of hydrolyzability such that, for example, one or more of the oligomers is rapidly removed from the drug by hydrolysis in the body and one or more of the oligomers is slowly removed from the drug by hydrolysis in the body.

The oligomer may be coupled to the drug at various nucleophilic residues of the drug including, but not limited to, nucleophilic hydroxyl functions and/or amino functions. When the drug is a polypeptide, a nucleophilic hydroxyl function may be found, for example, at serine and/or tyrosine residues, and a nucleophilic amino function may be found, for example, at histidine and/or lysine residues, and/or at the one or more N-termini of the

polypeptide. When an oligomer is coupled to the one or more N-termini of the insulin polypeptide, the coupling preferably forms a secondary amine. When the drug is human insulin, for example, the oligomer may be coupled to an amino functionality of the insulin, including the amino functionality of Gly^{A1}, the amino functionality of Phe^{B1}, and the amino functionality of Lys^{B29}. When one oligomer is coupled to the human insulin, the oligomer is preferably coupled to the amino functionality of Lys^{B29}. When two oligomers are coupled to the human insulin, the oligomers are preferably coupled to the amino functionality of Phe^{B1} and the amino functionality of Lys^{B29}. While more than one oligomer may be coupled to the human insulin, a higher activity (improved glucose lowering ability) is observed for the mono-conjugated human insulin. Monoconjugates (i.e., when one oligomer is coupled to the insulin drug) are preferably prepared using methods described in U.S. Patent Application Serial No. 10/036,744, entitled "Methods of Synthesizing Insulin Polypeptide-Oligomer Conjugates, and Proinsulin Polypeptide-Oligomer Conjugates and Methods of Synthesizing Same," the disclosure of which is incorporated herein by reference in its entirety. When the calcitonin drug is salmon calcitonin, for example, the oligomer may be coupled to an amino functionality of the salmon calcitonin, including the amino functionality of Lys^{11}, Lys^{18} and/or the N-terminus. While one or more oligomers may be coupled to the salmon calcitonin, a higher bioefficacy, such as improved serum calcium lowering ability, is observed for the di-conjugated salmon calcitonin where an oligomer is coupled to the amino functionalities of Lys^{11} and the Lys^{18}.

According to other embodiments of the present invention, a pharmaceutical composition comprises a drug-oligomer conjugate, a fatty acid component, and a bile salt component. The drug-oligomer conjugate includes a drug covalently coupled to an oligomeric moiety. The fatty acid component includes a fatty acid, and the bile salt component includes a bile salt.

The drug-oligomer conjugate includes a drug covalently coupled to an oligomeric moiety, and is similar to the drug-oligomer conjugates described above. The fatty acid component and the bile salt component are present in a weight-to-weight ratio of between 1:5 and 5:1, are preferably present in a weight-to-weight ratio of between 1:3 and 3:1, and are more preferably in a weight-to-weight ratio of between 1:2 and 2:1. The fatty acid component is similar to the fatty acid components described above, and the bile salt component is similar to the bile salt components described above.

According to these embodiments of the present invention, the fatty acid component is present in a first amount such that, at the precipitation point of the bile salt, the bile salt

precipitates as first bile salt particles that, upon a return to a pH above the precipitation point of the bile salt, re-solubilize more quickly than second bile salt particles that would have precipitated if the fatty acid component were present in a second amount less than the first amount. The precipitation point of the bile salt in the formulation is preferably at or below a pH of 6.0, and is more preferably at or below a pH of 5.5. The pH above the precipitation point may be various pH's above the precipitation point. In some embodiments, the pH above the precipitation point is at least 0.5 pH units above the precipitation point. In other embodiments, the pH above the precipitation point is at least 0.8 pH units above the precipitation point.

In some embodiments, the first bile salt particles have an average diameter of less than 500 microns and the second bile salt particles have an average diameter of greater than 550 microns. In other embodiments, the first bile salt particles have an average diameter of less than 100 microns and the second bile salt particles have an average diameter of greater than 150 microns.

In some embodiments, the first bile salt particles are able to re-solubilize in less than 75% of the time it would have taken for the second bile salt particles to re-solubilize. In other embodiments, the first bile salt particles are able to re-solubilize in less than half the time it would have taken for the second bile salt particles to re-solubilize.

According to still other embodiments of the present invention, a pharmaceutical composition comprises a drug-oligomer conjugate, between 0.1 and 15% (w/v) of a fatty acid component, and between 0.1 and 15% (w/v) of a bile salt component.

The drug-oligomer conjugate includes a drug covalently coupled to an oligomeric moiety, and is similar to the drug-oligomer conjugates described above. The fatty acid component and the bile salt component are present in a weight-to-weight ratio of between 1:5 and 5:1, are preferably present in a weight-to-weight ratio of between 1:3 and 3:1, and are more preferably present in a weight-to-weight ratio of between 1:2 and 2:1. The fatty acid component is similar to the fatty acid components described above, and the bile salt component is similar to the bile salt components described above.

According to these embodiments of the present invention, the concentration of the fatty acid component is between a lower limit of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14% (w/v) and an upper limit of 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 % (w/v). The concentration of the fatty acid component is preferably between 0.5 and 10% (w/v), is more preferably between 0.5 and 5% (w/v), and is still more preferably between 1 and 3% (w/v).

According to these embodiments of the present invention, the concentration of the bile acid component is between a lower limit of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14% (w/v) and an upper limit of 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 % (w/v). The concentration of the bile salt component is preferably between 0.5 and 10% (w/v), is more preferably between 1 and 5% (w/v), and is still more preferably between 2 and 4% (w/v).

Pharmaceutical compositions according to the present invention may further comprise a buffering component. The buffering component may comprise various buffering agents as will be understood by those skilled in the art. Exemplary buffering agents include, but are not limited to, inorganic acids (e.g., phosphoric acid), organic acids (e.g., citric acid), organic bases (e.g., tris-base (tris(hydroxymethyl)aminomethane), trolamine (triethanolamine), or histadine), and mixtures thereof. The buffering component preferably comprises an organic base, and more preferably comprises tris-base, trolamine, or a mixture thereof. In some embodiments, the buffering component comprises an organic acid and an organic base, and preferably comprises citric acid and tris-base, trolamine, or a mixture thereof. The buffering agent is preferably present in an amount that will buffer the pharmaceutical composition against the acidic environment that may be experienced in the gut as will be understood by one skilled in the art.

In addition to the bile salt component and fatty acid component, pharmaceutical compositions according to embodiments of the present invention may include various suitable excipients as will be understood by those skilled in the art, such as those found in the National Formulary 19, pages 2404-2406 (2000), the disclosure of pages 2404 to 2406 being incorporated herein in their entirety. For example, the pharmaceutical compositions may include lubricating agents such as, for example, talc, magnesium stearate and mineral oil; wetting agents; emulsifying and suspending agents; binding agents such as starches, gum arabic, microcrystalline cellulose, cellulose, methylcellulose, and syrup; anticaking agents such as calcium silicate; coating agents such as methacrylates and shellac; preserving agents such as methyl- and propyl hydroxybenzoates; sweetening agents; or flavoring agents. Polyols, and inert fillers may also be used. Examples of polyols include, but are not limited to, mannitol, sorbitol, xylitol, sucrose, maltose, glucose, lactose, dextrose, and the like. Other inert fillers which may be used encompass those which are known in the art and are useful in the manufacture of various dosage forms. If desired, the solid formulations may include other components such as bulking agents and/or granulating agents, and the like. The drug products of the invention may be formulated so as to provide quick, sustained, or delayed

release of the active ingredient after administration to the patient by employing procedures well known in the art.

The pharmaceutical compositions according to embodiments of the present invention include those suitable for oral, rectal, topical, inhalation (e.g., via an aerosol) buccal (e.g., sub-lingual), vaginal, parenteral (e.g., subcutaneous, intramuscular, intradermal, intraarticular, intrapleural, intraperitoneal, inracerebral, intraarterial, or intravenous), topical (i.e., both skin and mucosal surfaces, including airway surfaces) and transdermal administration, although the most suitable route in any given case will depend on the nature and severity of the condition being treated and on the nature of the particular drug-oligomer conjugate which is being used.

Pharmaceutical compositions suitable for oral administration may be presented in discrete units, such as capsules, cachets, lozenges, or tables, each containing a predetermined amount of the drug-oligomer conjugates; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. Such formulations may be prepared by any suitable method of pharmacy which includes the step of bringing into association the drug-oligomer conjugate, the fatty acid component, the bile salt component, and a suitable carrier (which may contain one or more accessory ingredients as noted above). In general, the pharmaceutical composition according to embodiments of the present invention are prepared by uniformly and intimately admixing the drug-oligomer conjugate, the fatty acid component, and the bile salt component with a liquid or finely divided solid carrier, or both, and then, if necessary, shaping the resulting mixture.

In some embodiments of the present invention, the pharmaceutical composition is a liquid pharmaceutical composition suitable for oral administration. When the pharmaceutical composition is a liquid pharmaceutical composition, the composition preferably includes a buffering agent as described above. Liquid pharmaceutical compositions according to embodiments of the present invention have a pH that is physiologically compatible. Preferably, liquid pharmaceutical compositions according to embodiments of the present invention have a pH that is between 6.2 and 9.0. In some embodiments, liquid pharmaceutical compositions according to embodiments of the present invention have a pH that is between a lower limit of 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, or 7.7 and an upper limit of 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, or 8.9. In some embodiments, liquid pharmaceutical compositions according to embodiments of the present invention have a pH that is between 7.0 and 8.5. In

other embodiments, liquid pharmaceutical compositions according to embodiments of the present invention have a pH that is between 7.4 and 8.2.

In other embodiments of the present invention, the pharmaceutical composition is a solid pharmaceutical composition suitable for oral administration. The solid pharmaceutical composition may be prepared by various methods as will be understood by those skilled in the art. For example, a tablet may be prepared by compressing or molding a powder or granules containing the drug-oligomer conjugate, the fatty acid component, the bile salt component, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine, the mixture in a free-flowing form, such as a powder or granules optionally mixed with a binder, lubricant, inert diluent, and/or surface active/dispersing agent(s). Molded tablets may be made by molding, in a suitable machine, the powdered compound moistened with an inert liquid binder.

Pharmaceutical compositions suitable for buccal (sub-lingual) administration include lozenges comprising the drug-oligomer conjugate, the fatty acid component, and the bile salt component in a flavored base, usually an artificial sweetener and acacia or tragacanth; and pastilles comprising the drug-oligomer conjugate, the fatty acid component, and the bile salt component in an inert base such as gelatin and glycerin or sucrose and acacia.

Pharmaceutical compositions according to embodiments of the present invention suitable for parenteral administration comprise sterile aqueous and non-aqueous injection solutions comprising the drug-oligomer conjugate, the fatty acid component, and the bile salt component, which preparations are preferably isotonic with the blood of the intended recipient. These preparations may contain anti-oxidants, buffers, bacteriostats and solutes which render the composition isotonic with the blood of the intended recipient. Aqueous and non-aqueous sterile suspensions may include suspending agents and thickening agents. The compositions may be presented in unit\dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline or water-for-injection immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described. For example, an injectable, stable, sterile composition comprising the drug-oligomer conjugate, the fatty acid component, and the bile salt component in a unit dosage form in a sealed container may be provided. The mixture of the drug-oligomer conjugate, the fatty acid component, and the bile salt component is provided in the form of a lyophilizate which is capable of being reconstituted with a suitable pharmaceutically acceptable carrier to form a

liquid composition suitable for injection thereof into a subject. The unit dosage form typically comprises from about 10 mg to about 10 grams of the drug-oligomer conjugate. When the drug-oligomer conjugate is substantially water-insoluble, a sufficient amount of emulsifying agent which is physiologically acceptable may be employed in sufficient quantity to emulsify the drug-oligomer conjugate in an aqueous carrier. One such useful emulsifying agent is phosphatidyl choline.

Pharmaceutical compositions suitable for rectal administration are preferably presented as unit dose suppositories. These may be prepared by admixing the drug-oligomer conjugate, the fatty acid component, and the bile salt component with one or more conventional solid carriers, for example, cocoa butter, and then shaping the resulting mixture.

Pharmaceutical compositions suitable for topical application to the skin preferably take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers which may be used include petroleum jelly, lanoline, polyethylene glycols, alcohols, transdermal enhancers, and combinations of two or more thereof.

Pharmaceutical compositions suitable for transdermal administration may be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Compositions suitable for transdermal administration may also be delivered by iontophoresis *(see,* for example, *Pharmaceutical Research* 3 (6):318 (1986)) and typically take the form of an optionally buffered aqueous solution of the drug-oligomer conjugate, the fatty acid component, and the bile salt component. Suitable formulations comprise citrate or bis\tris buffer (pH 6) or ethanol/water and contain from 0.1 to 0.2M active ingredient.

Methods of treating an insulin deficieny in a subject in need of such treatment by administering any of the various pharmaceutical compositions described above that contain a therapeutically effective amount of an insulin drug-oligomer conjugate are also provided. The effective amount of the insulin drug-oligomer conjugate, the use of which is in the scope of present invention, will vary somewhat from conjugate to conjugate, and subject to subject, and will depend upon factors such as the age and condition of the subject and the route of delivery. Such dosages can be determined in accordance with routine pharmacological procedures known to those skilled in the art. As a general proposition, a dosage from about 0.1 to about 50 mg/kg will have therapeutic efficacy, with all weights being calculated based upon the weight of the insulin drug-oligomer conjugate. Toxicity concerns at the higher level may restrict intravenous dosages to a lower level such as up to about 10 mg/kg, with all weights being calculated based upon the weight of the active base. A dosage from about 10

mg/kg to about 50 mg/kg may be employed for oral administration. Typically, a dosage from about 0.5 mg/kg to 5 mg/kg may be employed for intramuscular injection. The frequency of administration is usually one, two, or three times per day or as necessary to control the condition. Alternatively, the drug-oligomer conjugates may be administered by continuous infusion. The duration of treatment depends on the type of insulin deficiency being treated and may be for as long as the life of the subject.

Methods of treating a bone disorder in a subject in need of such treatment by administering any of the various pharmaceutical compositions described above that contain a therapeutically effective amount of a calcitonin drug-oligomer conjugate are also provided. The bone disorder is preferably characterized by excessive osteoclastic bone resorption and/or hypercalcemic serum effects. Bone disorders that may be treated and/or prevented by methods of the present invention include, but are not limited to, osteoporosis, Paget's disease, and hypercalcemia.

The effective amount of the calcitonin drug-oligomer conjugate, the use of which is in the scope of present invention, will vary somewhat from conjugate to conjugate, and patient to patient, and will depend upon factors such as the age and condition of the patient and the route of delivery. Such dosages can be determined in accordance with routine pharmacological procedures known to those skilled in the art. As a general proposition, a dosage from about 0.1 to about 50 mg/kg will have therapeutic efficacy, with all weights being calculated based upon the weight of the mixture of calcitonin drug-oligomer conjugates. Toxicity concerns at the higher level may restrict intravenous dosages to a lower level such as up to about 10 mg/kg, with all weights being calculated based upon the weight of the active base. A dosage from about 10 mg/kg to about 50 mg/kg may be employed for oral administration. Typically, a dosage from about 0.5 mg/kg to 5 mg/kg may be employed for intramuscular injection. The frequency of administration is usually one, two, or three times per day or as necessary to control the condition. Alternatively, the drug-oligomer conjugates may be administered by continuous infusion. The duration of treatment depends on the type of bone disorder being treated and may be for as long as the life of the patient.

In another aspect of the present invention, a method of providing a pharmaceutical composition comprises selecting an amount of a bile salt to include in the composition based on the ability of the bile salt to increase the solubility of a fatty acid component when the composition has a pH of 8.5 or less.

According to other embodiments of the present invention, a method of providing a pharmaceutical composition comprises selecting an amount of a fatty acid to include in the

composition based on the ability of the fatty acid to lower the precipitation point of a bile salt component in the composition to a pH of 5.5 or less.

According to still other embodiments of the present invention, a method of providing a pharmaceutical composition comprises selecting an amount of a fatty acid to include in the composition based on the ability of the fatty acid to alter the precipitation characteristics of a bile salt component in the composition.

The present invention will now be described with reference to the following examples. It should be appreciated that these examples are for the purposes of illustrating aspects of the present invention, and do not limit the scope of the invention as defined by the claims.

Examples

Example 1

Synthesis of 6-(2-{2-[2-(2-{2-[2-(2-methoxyethoxy)ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethoxy}-ethoxy)-hexanoic acid 2,5-dioxo-pyrrolidin-1-yl ester (8)

Hexaethylene glycol monobenzyl ether (1). An aqueous sodium hydroxide solution prepared by dissolving 3.99 g (100 mmol) NaOH in 4 ml water was added slowly to monodispersed hexaethylene glycol (28.175 g, 25 ml, 100 mmol). Benzyl chloride (3.9 g, 30.8 mmol, 3.54 ml) was added and the reaction mixture was heated with stirring to 100°C for 18 hours. The reaction mixture was then cooled, diluted with brine (250 ml) and extracted with methylene chloride (200 ml x 2). The combined organic layers were washed with brine once, dried over Na_2SO_4, filtered and concentrated in vacuo to a dark brown oil. The crude product mixture was purified *via* flash chromatography (silica gel, gradient elution: ethyl acetate to 9/1 ethyl acetate/methanol) to yield 8.099 g (70 %) of monodispersed compound **1** as a yellow oil.

Ethyl 6-methylsulfonyloxyhexanoate (2). A solution of monodispersed ethyl 6-hydroxyhexanoate (50.76 ml, 50.41 g, 227 mmol) in dry dichloromethane (75 ml) was chilled in an ice bath and placed under a nitrogen atmosphere. Triethylamine (34.43 ml, 24.99 g, 247 mmol) was added. A solution of methanesulfonyl chloride (19.15 ml, 28.3 g, 247 mmol) in dry dichloromethane (75 ml) was added dropwise from an addition funnel. The mixture

was stirred for three and one half hours, slowly being allowed to come to room temperature as the ice bath melted. The mixture was filtered through silica gel, and the filtrate was washed successively with water, saturated $NaHCO_3$, water and brine. The organics were dried over Na_2SO_4, filtered and concentrated in vacuo to a pale yellow oil. Final purification of the crude product was achieved by flash chromatography (silica gel, 1/1 hexanes/ethyl acetate) to give the monodispersed compound **2** (46.13 g, 85 %) as a clear, colorless oil. FAB MS: *m/e* 239 (M+H), 193 ($M-C_2H_5O$).

6-{2-[2-(2-{2-[2-(2-Benzyloxyethoxy)ethoxy]ethoxy}-ethoxy)- ethoxy]-ethoxy}-hexanoic acid ethyl ester (3). Sodium hydride (3.225 g or a 60 % oil dispersion, 80.6 mmol) was suspended in 80 ml of anhydrous toluene, placed under a nitrogen atmosphere and cooled in an ice bath. A solution of the monodispersed alcohol **9** (27.3 g, 73.3 mmol) in 80 ml dry toluene was added to the NaH suspension. The mixture was stirred at 0°C for thirty minutes, allowed to come to room temperature and stirred for another five hours, during which time the mixture became a clear brown solution. The monodispersed mesylate **10** (19.21 g, 80.6 mmol) in 80 ml dry toluene was added to the NaH/alcohol mixture, and the combined solutions were stirred at room temperature for three days. The reaction mixture was quenched with 50 ml methanol and filtered through basic alumina. The filtrate was concentrated in vacuo and purified by flash chromatography (silica gel, gradient elution: 3/1 ethyl acetate/hexanes to ethyl acetate) to yield the monodispersed compound **3** as a pale yellow oil (16.52 g, 44 %). FAB MS: *m/e* 515 (M+H).

6-{2-[2-(2-{2-[2-(2-hydroxyethoxy)ethoxy]ethoxy}-ethoxy)-ethoxy]-ethoxy}-hexanoic acid ethyl ester (4). Substantially monodispersed benzyl ether **3** (1.03 g, 2.0 mmol) was dissolved in 25 ml ethanol. To this solution was added 270 mg 10 % Pd/C, and the mixture was placed under a hydrogen atmosphere and stirred for four hours, at which time TLC showed the complete disappearance of the starting material. The reaction mixture was filtered through Celite 545 to remove the catalyst, and the filtrate was concentrated in vacuo to yield the monodispersed compound **4** as a clear oil (0.67 g, 79 %). FAB MS: *m/e* 425 (M+H), 447 (M+Na).

6-{2-[2-(2-{2-[2-(2-methylsulfonylethoxy)ethoxy]ethoxy}-ethoxy)-ethoxy]-ethoxy}-hexanoic acid ethyl ester (5). The monodispersed alcohol **4** (0.835 g, 1.97 mmol) was dissolved in 3.5 ml dry dichloromethane and placed under a nitrogen atmosphere.

Triethylamine (0.301 ml, 0.219 g, 2.16 mmol) was added and the mixture was chilled in an ice bath. After two minutes, the methanesulfonyl chloride (0.16 ml, 0.248 g, 2.16 mmol) was added. The mixture was stirred for 15 minutes at $0^{\circ}C$, then at room temperature for two hours. The reaction mixture was filtered through silica gel to remove the triethylammonium chloride, and the filtrate was washed successively with water, saturated $NaHCO_3$, water and brine. The organics were dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 9/1 ethyl acetate/methanol) to give monodispersed compound **5** as a clear oil (0.819 g, 83 %). FAB MS: *m/e* 503 (M+H).

6-(2-{2-[2-(2-{2-[2-(2-methoxyethoxy)ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethoxy}-ethoxy)-hexanoic acid ethyl ester (6). NaH (88 mg of a 60 % dispersion in oil, 2.2 mmol) was suspended in anhydrous toluene (3 ml) under N_2 and chilled to $0^{\circ}C$. Monodispersed diethylene glycol monomethyl ether (0.26 ml, 0.26 g, 2.2 mmol) that had been dried *via* azeotropic distillation with toluene was added. The reaction mixture was allowed to warm to room temperature and stirred for four hours, during which time the cloudy grey suspension became clear and yellow and then turned brown. Mesylate **5** (0.50 g, 1.0 mmol) in 2.5 ml dry toluene was added. After stirring at room temperature over night, the reaction was quenched by the addition of 2 ml of methanol and the resultant solution was filtered through silica gel. The filtrate was concentrated in vacuo and the FAB MS: *m/e* 499 (M+H), 521 (M+Na). Additional purification by preparatory chromatography (silica gel, 19/3 chloroform/methanol) provided the monodispersed compound **6** as a clear yellow oil (0.302 g 57 %). FAB MS: *m/e* 527 (M+H), 549 (M+Na).

6-(2-{2-[2-(2-{2-[2-(2-methoxyethoxy)ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethoxy}-ethoxy)-hexanoic acid (7). Monodispersed ester **6** (0.25 g, 0.46 mmol) was stirred for 18 hours in 0.71 ml of 1 N NaOH. After 18 hours, the mixture was concentrated in vacuo to remove the alcohol and the residue dissolved in a further 10 ml of water. The aqueous solution was acidified to pH 2 with 2 N HCl and the product was extracted into dichloromethane (30 ml x 2). The combined organics were then washed with brine (25 ml x 2), dried over Na_2SO_4, filtered and concentrated in vacuo to yield the monodispersed compound **15** as a yellow oil (0.147 g, 62 %). FAB MS: *m/e* 499 (M+H), 521 (M+Na).

6-(2-{2-[2-(2-{2-[2-(2-methoxyethoxy)ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethoxy}-ethoxy)-hexanoic acid 2,5-dioxo-pyrrolidin-1-yl ester (8). Monodispersed acid 7 (0.209 g, 0.42 mmol) was dissolved in 4 ml of dry dichloromethane and added to a dry flask already containing NHS (N-hydroxysuccinimide) (57.8 mg, 0.502 mmol) and EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) (98.0 mg, 0.502 mmol) under a N_2 atmosphere. The solution was stirred at room temperature overnight and filtered through silica gel to remove excess reagents and the urea formed from the EDC. The filtrate was concentrated in vacuo to provide the activated monodispersed oligomer **8** as a dark yellow oil (0.235 g, 94 %). FAB MS: *m/e* 596 (M+H), 618 (M+Na).

Example 2

Synthesis of Activated MPEG$_7$-C$_8$ (14)

Mesylate of triethylene glycol monomethyl ether (9). To a solution of CH_2Cl_2 (100 mL) cooled to 0°C in an ice bath was added monodispersed triethylene glycol monomethyl ether (25 g, 0.15 mol). Then triethylamine (29.5 mL, 0.22 mol) was added and the solution was stirred for 15 min at 0°C, which was followed by dropwise addition of methanesulfonyl chloride (13.8 mL, 0.18 mol, dissolved in 20 mL CH_2Cl_2). The reaction mixture was stirred for 30 min at 0°C, allowed to warm to room temperature, and then stirred for 2 h. The crude reaction mixture was filtered through Celite (washed CH_2Cl_2 ~200 mL), then washed with H_2O (300 mL), 5% $NaHCO_3$ (300 mL), H_2O (300 mL), sat. NaCl (300 mL), dried $MgSO_4$, and evaporated to dryness. The oil was then placed on a vacuum line for ~2h to ensure dryness and afforded the monodispersed compound **9** as a yellow oil (29.15 g, 80% yield).

Heptaethylene glycol monomethyl ether (10). To a solution of monodispersed tetraethylene glycol (51.5 g, 0.27 mol) in THF (1L) was added potassium t-butoxide (14.8 g, 0.13 mol, small portions over ~30 min). The reaction mixture was then stirred for 1h and then **9** (29.15 g, 0.12 mol) dissolved in THF (90 mL) was added dropwise and the reaction mixture was stirred overnight. The crude reaction mixture was filtered through Celite (washed CH_2Cl_2, ~200 mL) and evaporated to dryness. The oil was then dissolved in HCl (250 mL, 1 N) and washed with ethyl acetate (250 mL) to remove excess **9**. Additional washings of ethyl acetate (125 mL) may be required to remove remaining **9**. The aqueous phase was washed repetitively with CH_2Cl_2 (125 mL volumes) until most of the compound **18** has been removed from the aqueous phase. The first extraction will contain **9**, **10**, and

dicoupled side product and should be back extracted with HCl (125 mL, 1N). The organic layers were combined and evaporated to dryness. The resultant oil was then dissolved in CH_2Cl_2 (100 mL) and washed repetitively with H_2O (50 mL volumes) until **10** was removed. The aqueous fractions were combined, total volume 500 mL, and NaCl was added until the solution became cloudy and then was washed with CH_2Cl_2 (2 x 500 mL). The organic layers were combined, dried $MgSO_4$, and evaporated to dryness to afford the monodispersed compound **10** as an oil (16.9 g, 41% yield). It may be desirable to repeat one or more steps of the purification procedure to ensure high purity.

8-Bromooctoanate (11). To a solution of monodispersed 8-bromooctanoic acid (5.0 g, 22 mmol) in ethanol (100 mL) was added H_2SO_4 (0.36 mL, 7.5 mmol) and the reaction was heated to reflux with stirring for 3 h. The crude reaction mixture was cooled to room temperature and washed H_2O (100 mL), sat. $NaHCO_3$ (2 x 100 mL), H_2O (100 mL), dried $MgSO_4$, and evaporated to dryness to afford a clear oil **11** (5.5 g, 98% yield).

MPEG$_7$-C$_8$ ester (12). To a solution of the monodispersed compound **10** (3.0 g, 8.8 mmol) in ether (90 mL) was added potassium t-butoxide (1.2 g, 9.6 mmol) and the reaction mixture was stirred for 1 h. Then dropwise addition of the monodispersed compound **11** (2.4 g, 9.6 mmol), dissolved in ether (10 mL), was added and the reaction mixture was stirred overnight. The crude reaction mixture was filtered through Celite (washed CH_2Cl_2, ~200 mL) and evaporated to dryness. The resultant oil was dissolved in ethyl acetate and washed H_2O (2 x 200 mL), dried $MgSO_4$, and evaporated to dryness. Column chromatography (Silica, ethyl acetate to ethyl acetate/methanol, 10:1) was performed and afforded the monodispersed compound **12** as a clear oil (0.843 g, 19% yield).

MPEG$_7$-C$_8$ acid (13). To the oil of the monodispersed compound **12** (0.70 g, 1.4 mmol) was added 1N NaOH (2.0 mL) and the reaction mixture was stirred for 4h. The crude reaction mixture was concentrated, acidified (pH~2), saturated with NaCl, and washed CH_2Cl_2 (2 x 50 mL). The organic layers were combined, washed sat. NaCl, dried $MgSO_4$, and evaporated to dryness to afford the monodispersed compound **13** as a clear oil (0.35 g, 53% yield).

Activation of MPEG$_7$-C$_8$ acid. Monodispersed mPEG7-C8-acid **13** (0.31g, 0.64 mmol) was dissolved in 3 ml of anhydrous methylene chloride and then solution of N-

hydroxysuccinimide (0.079g, 0.69 mmol) and EDCI·HCl (135.6 mg, 0.71 mmol) in anhydrous methylene chloride added. Reaction was stirred for several hours, then washed with 1N HCl, water, dried over $MgSO_4$, filtered and concentrated. Crude material was purified by column chromatography, concentrated to afford monodispersed activated $MPEG_7$-C_8 **14** as a clear oil and dried *via* vacuum.

Example 3

Synthesis of Activated $MPEG_7$-C_{10} (19)

10-hydroxydecanoate (15). To a solution of monodispersed 10-hydroxydecanoic acid (5.0 g, 26.5 mmol) in ethanol (100 mL) was added H_2S0_4 (0.43 mL, 8.8 mmol) and the reaction was heated to reflux with stirring for 3 h. The crude reaction mixture was cooled to room temperature and washed H_2O (100 mL), sat. $NaHCO_3$ (2 x 100 mL), H_20 (100 mL), dried $MgSO_4$, and evaporated to dryness to afford the monodispersed compound **15** as a clear oil (6.9 g, 98% yield).

Mesylate of 10-hydroxydecanoate (16). To a solution of CH_2Cl_2 (27 mL) was added monodispersed 10-hydroxydecanoate **15** (5.6 g, 26 mmol) and cooled to 0°C in an ice bath. Then triethylamine (5 mL, 37 mmol) was added and the reaction mixture was stirred for 15 min at 0°C. Then methanesulfonyl chloride (2.7 mL, 24 mmol) dissolved in CH_2Cl_2 (3 mL) was added and the reaction mixture was stirred at 0°C for 30 min, the ice bath was removed and the reaction was stirred for an additional 2 h at room temperature. The crude reaction mixture was filtered through Celite (washed CH_2C1_2, 80 mL) and the filtrate was washed H_2O (100 mL), 5% $NaHCO_3$ (2 x 100 mL), H_2O (100 mL), sat. NaCl (100 mL), dried $MgSO_4$, and evaporated to dryness to afford the monodispersed compound **16** as a yellowish oil (7.42 g, 97% yield).

$MPEG_7$-C_{10} Ester (17). To a solution of substantially monodispersed heptaethylene glycol monomethyl ether **10** (2.5 g, 7.3 mmol) in tetrahydrofuran (100 mL) was added sodium hydride (0.194 g, 8.1 mmol) and the reaction mixture was stirred for 1 h. Then dropwise addition of mesylate of monodispersed 10-hydroxydecanoate **16** (2.4 g, 8.1 mmol), dissolved in tetrahydrofuran (10 mL), was added and the reaction mixture was stirred overnight. The crude reaction mixture was filtered through Celite (washed CH_2C1_2, ~200 mL) and evaporated to dryness. The resultant oil was dissolved in ethyl acetate and washed H_2O (2 x 200 mL), dried $MgSO_4$, evaporated to dryness, chromatographed (silica, ethyl

acetate/methanol, 10:1), and chromatographed (silica, ethyl acetate) to afford the monodispersed compound **17** as a clear oil (0.570 g, 15% yield).

MPEG$_7$-C$_{10}$ Acid (18). To the oil of monodispersed mPEG$_7$-C$_{10}$ ester **17** (0.570 g, 1.1 mmol) was added 1N NaOH (1.6 mL) and the reaction mixture was stirred overnight. The crude reaction mixture was concentrated, acidified (pH~2), saturated with NaCl, and washed CH_2Cl_2 (2 x 50 mL). The organic layers were combined, washed sat. NaCl (2 x 50 mL), dried $MgSO_4$, and evaporated to dryness to afford the monodispersed compound **18** as a clear oil (0.340 g, 62% yield).

Activation of MPEG$_7$-C$_{10}$ Acid. The monodispersed acid **18** was activated using procedures similar to those described above in Example 10 to provide activated MPEG$_7$-C$_{10}$ Oligomer **19**.

Example 4

Synthesis of Activated C$_{18}$(PEG$_6$) Oliomer (22)

Synthesis of C$_{18}$(PEG$_6$) Oligomer (20). Monodispersed stearoyl chloride (0.7g, 2.31 mmol) was added slowly to a mixture of monodispersed PEG$_6$ (5 g, 17.7 mmol) and pyridine (0.97g, 12.4 mmol) in benzene. The reaction mixture was stirred for several hours (~5). The reaction was followed by TLC using ethylacetate/methanol as a developing solvent. Then the reaction mixture was washed with water, dried over $MgSO_4$, concentrated and dried *via* vacuum. Purified monodispersed compound **20** was analyzed by FABMS: m/e 549/ M^+H.

Activation of C$_{18}$(PEG$_6$) Oligomer. Activation of monodispersed C$_{18}$(PEG$_6$) oligomer was accomplished in two steps:

1) Monodispersed stearoyl-PEG$_6$ **20** (0.8 g, 1.46 mmol) was dissolved in toluene and added to a phosgene solution (10 ml, 20 % in toluene) which was cooled with an ice bath. The reaction mixture was stirred for 1 h at 0°C and then for 3 h at room temperature. Then phosgene and toluene were distilled off and the remaining substantially monodispersed stearoyl PEG6 chloroformate **21** was dried over P_2O_5 overnight.

2) To a solution of monodispersed stearoyl-PEG$_6$ chloroformate **21** (0.78g, 1.27 mmol) and TEA (128 mg, 1.27 mmol) in anhydrous methylene chloride, N-hydroxy succinimide (NHS) solution in methylene chloride was added. The reaction mixture was

stirred for 16 hours, then washed with water, dried over $MgSO_4$, filtered, concentrated and dried *via* vacuum to provide the monodispersed activated $C_{18}(PEG_6)$ oligomer **22.**

Example 5

Synthesis of Activated $C_{18}(PEG_8)$ Oligomer (28)

Tetraethylene glycol monobenzylether (23). To the oil of monodispersed tetraethylene glycol (19.4 g, 0.10 mol) was added a solution of NaOH (4.0 g in 4.0 mL) and the reaction was stirred for 15 min. Then benzyl chloride (3.54 mL, 30.8 mmol) was added and the reaction mixture was heated to 100°C and stirred overnight. The reaction mixture was cooled to room temperature, diluted with sat. NaCl (250 mL), and washed CH_2Cl_2 (2 x 200 mL). The organic layers were combined, washed sat. NaCl, dried $MgSO_4$, and chromatographed (silica, ethyl acetate) to afford the monodispersed compound **23** as a yellow oil (6.21 g, 71% yield).

Mesylate of tetraethylene glycol monobenzylether (24). To a solution of CH_2CI_2 (20 mL) was added monodispersed tetraethylene glycol monobenzylether **23** (6.21 g, 22 mmol) and cooled to 0°C in an ice bath. Then triethylamine (3.2 mL, 24 mmol) was added and the reaction mixture was stirred for 15 min at 0°C. Then methanesulfonyl chloride (1.7 mL, 24 mmol) dissolved in CH_2CI_2 (2 mL) was added and the reaction mixture was stirred at 0°C for 30 min, the ice bath was removed and the reaction was stirred for an additional 2 h at room temperature. The crude reaction mixture was filtered through Celite (washed CH_2CI_2, 80 mL) and the filtrate was washed H_2O (100 mL), 5% $NaHCO_3$ (2 x 100 mL), H_20 (100 mL), sat. NaCl (100 mL), and dried $MgSO_4$. The resulting yellow oil was chromatographed on a pad of silica containing activated carbon (10 g) to afford the monodispersed compound **24** as a clear oil (7.10 g, 89% yield).

Octaethylene glycol monobenzylether (25). To a solution of tetrahydrofuran (140 mL) containing sodium hydride (0.43 g, 18 mmol) was added dropwise a solution of monodispersed tetraethylene glycol (3.5 g, 18 mmol) in tetrahydrofuran (10 mL) and the reaction mixture was stirred for 1 h. Then mesylate of monodispersed tetraethylene glycol monobenzylether **24** (6.0 g, 16.5 mmol) dissolved in tetrahydrofuran (10 mL) was added dropwise and the reaction mixture was stirred overnight. The crude reaction mixture was filtered through Celite (washed, CH_2Cl_2, 250 mL) and the filtrate was washed H_2O, dried $MgSO_4$, and evaporated to dryness. The resultant oil was chromatographed (silica, ethyl

acetate/methanol, 10:1) and chromatographed (silica, chloroform/methanol, 25:1) to afford the monodispersed compound **25** as a clear oil (2.62 g, 34% yield).

Synthesis of Stearate PEG_8-Benzyl (26). To a stirred cooled solution of monodispersed octaethylene glycol monobenzylether **25** (0.998 g, 2.07 mmol) and pyridine (163.9 mg, 2.07 mmol) was added monodispersed stearoyl chloride (627.7 mg, 2.07 mmol) in benzene. The reaction mixture was stirred overnight (18 hours). The next day the reaction mixture was washed with water, dried over $MgSO_4$, concentrated and dried *via* vacuum. Then the crude product was chromatographed on flash silica gel column, using 10% methanol/90% chloroform. The fractions containing the product were combined, concentrated and dried *via* vacuum to afford the monodispersed compound **26**.

Hydrogenolysis of Stearate-PEG_8-Benzyl. To a methanol solution of monodispersed stearate-PEG_8-Bzl **26** (0.854g 1.138 mmol) Pd/C(10%) (palladium, 10% wt. on activated carbon) was added. The reaction mixture was stirred overnight (18 hours) under hydrogen. Then the solution was filtered, concentrated and purified by flash column chromatography using 10% methanol/90% chloroform, fractions with R_f=0.6 collected, concentrated and dried to provide the monodispersed acid **27**.

Activation of $C_{18}(PEG_8)$ Oligomer. Two step activation of monodispersed stearate-PEG8 oligomer **27** was performed as described for stearate-PEG_6 in Example 4 above to provide the monodispersed activated $C_{18}(PEG_8)$ oligomer **28**.

Example 6

Synthesis of Activated Triethylene Glycol Monomethyl Oligomers

A solution of toluene containing 20% phosgene (100 ml, approximately 18.7 g, 189 mmol phosgene) was chilled to 0°C under a N_2 atmosphere. Monodispersed mTEG (triethylene glycol, monomethyl ether, 7.8 g, 47.5 mmol) was dissolved in 25 mL anhydrous ethyl acetate and added to the chilled phosgene solution. The mixture was stirred for one hour at 0°C, then allowed to warm to room temperature and stirred for another two and one half hours. The remaining phosgene, ethyl acetate and toluene were removed *via* vacuum distillation to leave the monodispersed mTEG chloroformate as a clear oily residue.

The monodispersed nTEG chloroformate was dissolved in 50 mL of dry dichloromethane to which was added TEA (triethyleamine, 6.62 mL, 47.5 mmol) and NHS

(N-hydroxysuccinimide, 5.8 g, 50.4 mmol). The mixture was stirred at room temperature under a dry atmosphere for twenty hours during which time a large amount of white precipitate appeared. The mixture was filtered to remove this precipitate and concentrated *in vacuo*. The resultant oil was taken up in dichloromethane and washed twice with cold deionized water, twice with 1N HCl and once with brine. The organics were dried over $MgSO_4$, filtered and concentrated to provide the monodispersed title compound as a clear, light yellow oil. If necessary, the NHS ester could be further purified by flash chromatography on silica gel using EtOAc as the elutant.

Example 7

Synthesis of Activated Palmitate-TEG Oligomers

Monodispersed palmitic anhydride (5 g; 10 mmol) was dissolved in dry THF (20 mL) and stirred at room temperature. To the stirring solution, 3 mol excess of pyridine was added followed by monodispersed triethylene glycol (1.4 mL). The reaction mixture was stirred for 1 hour (progress of the reaction was monitored by TLC; ethyl acetate-chloroform; 3:7). At the end of the reaction, THF was removed and the product was mixed with 10% H_2SO_4 acid and extracted ethyl acetate (3 x 30 mL). The combined extract was washed sequentially with water, brine, dried over $MgSO_4$, and evaporated to give monodispersed palmitate-TEG oligomers.

A solution of N,N'-disuccinimidyl carbonate (3 mmol) in DMF (~10 mL) is added to a solution of the monodispersed palmitate-TEG oligomers (1 mmol) in 10 mL of anydrous DMF while stirring. Sodium hydride (3 mmol) is added slowly to the reaction mixture. The reaction mixture is stirred for several hours (e.g., 5 hours). Diethyl ether is added to precipitate the monodispersed activated title oligomer. This process is repeated 3 times and the product is finally dried.

Example 8

Synthesis of Activated Hexaethylene Glycol Monomethyl Oligomers

Monodispersed activated hexaethylene glycol monomethyl ether was prepared analogously to that of monodispersed triethylene glycol in Example 6 above. A 20% phosgene in toluene solution (35 mL, 6.66 g, 67.4 mmol phosgene) was chilled under a N_2 atmosphere in an ice/salt water bath. Monodispersed hexaethylene glycol (1.85 mL, 2.0 g, 6.74 mmol) was dissolved in 5 mL anhydrous EtOAc and added to the phosgene solution *via*

syringe. The reaction mixture was kept stirring in the ice bath for one hour, removed and stirred a further 2.5 hours at room temperature. The phosgene, EtOAc, and toluene were removed by vacuum distillation, leaving monodispersed methyl hexaethylene glycol chloroformate as a clear, oily residue.

The monodispersed chloroformate was dissolved in 20 mL dry dichloromethane and placed under a dry, inert atmosphere. Triethylamine (0.94 mL, 0.68 g, 6.7 mmol) and then NHS (N-hydroxy succinimide, 0.82 g, 7.1 mmol) were added, and the reaction mixture was stirred at room temperature for 18 hours. The mixture was filtered through silica gel to remove the white precipitate and concentrated *in vacuo*. The residue was taken up in dichloromethane and washed twice with cold water, twice with 1 N HCl and once with brine. The organics were dried over Na_2SO_4, filtered and concentrated. Final purification was done via flash chromatography (silica gel, EtOAc) to obtain the activated monodispersed hexaethylene monomethyl ether.

Example 9

Synthesis of Avtivated Heptaethylene Glycol Monomethyl Ether

8-Methoxy-1-(methylsulfonyl)oxy-3,6-dioxaoctane (29). A solution of monodispersed triethylene glycol monomethyl ether molecules (4.00 mL, 4.19 g, 25.5 mmol) and triethylamine (4.26 mL, 3.09 g, 30.6 mmol) in dry dichloromethane (50 mL) was chilled in an ice bath and place under a nitrogen atmosphere. A solution of methanesulfonyl chloride (2.37 mL, 3.51 g, 30.6 mmol) in dry dichloromethane (20 mL) was added dropwise from an addition funnel. Ten minutes after the completion of the chloride addition, the reaction mixture was removed from the ice bath and allowed to come to room temperature. The mixture was stirred for an additional hour, at which time TLC ($CHCl_3$ with 15% MeOH as the elutant) showed no remaining triethylene glycol monomethyl ether.

The reaction mixture was diluted with another 75 mL of dichloromethane and washed successively with saturated $NaHCO_3$, water and brine. The organics were dried over Na_2SO_4, filtered and concentrated in vacuo to give a monodispersed mixture of compounds **29** as a clear oil (5.31g, 86%).

Heptaethylene glycol mono methyl ether (30). To a stirred solution of monodispersed tetraethylene glycol (35.7 mmol) in dry DMF (25.7 mL), under N_2 was added in portion a 60% dispersion of NaH in mineral oil, and the mixture was stirred at room temperature for 1 hour. To the resulting sodium salt of the tetraethylene glycol was added a

solution of monodispersed mesylate **29** (23.36) in dry DMF (4 ml) in a single portion, and the mixture was stirred at room temperature for 3.5 hours. Progress of the reaction was monitored by TLC (12% CH_3OH-$CHCl_3$). The reaction mixture was diluted with an equal amount of 1N HCl, and extracted with ethyl acetate (2 x 20 ml) and discarded. Extraction of aqueous solution and work-up gave monodispersed heptaethylene glycol monomethyl ether **30** (82 -84% yield). Oil; Rf 0.46 (methanol : chloroform = 3:22); MS m/z calc'd for $C_{15}H_{32}O_8$ 340.21 (M^++1), found 341.2.

Activation of heptaethylene glycol monomethyl ether. Monodispersed heptaethylene glycol monomethyl ether **30** is activated by a procedure similar to that used in Example 6 above to activate triethylene glycol monomethyl ether to provide the activated heptaethylene glycol monomethyl ether.

Example 10

Synthesis of Activated Decaethylene Glycol Monomethyl Ether (33)

20-methoxy-1-(methylsulfonyl)oxy-3,6,9,12,15,18-hexaoxaeicosane (31). Monodispersed compound **31** was obtained in quantitative yield from compound **30** and methanesulfonyl chloride as described for **29** in Example 9 above, as an oil; Rf 0.4 (ethyl acetate : acetonitrile = 1:5); MS m/z calc'd for $C_{17}H_{37}O_{10}$ 433.21 (M^++1), found 433.469.

Decaethylene glycol monomethyl ether (32). Monodispersed compound **32** was prepared from compound **31** and monodispersed triethylene glycol using the procedure described above in Example 17. Oil; Rf 0.41 (methanol: chloroform = 6:10); MS m/z calc'd for $C_{21}H_{44}O_{11}$ 472.29 (M^++1), found 472.29.

Activation of decaethylene glycol monomethyl ether. Monodispersed decaethylene glycol monomethyl ether **32** is activated by a procedure similar to that used in Example 6 above to activate triethylene glycol monomethyl ether to provide the activated decaethylene glycol monomethyl ether **33**.

Example 11

HIM2 Oral Liquid Process

A general procedure to manufacture an oral liquid pharmaceutical composition of the present invention is shown below: The process involves making a premix without the drug, filtering the premix, then adding the premix and drug solution together.

Quantitative Composition of HIM2 Oral Liquid, 6 mg/mL

Excipient	Composition		Quantity per Batch (g)
	%w/v	mg/mL	
HIM2	0.6	6	6.0[1]
Sodium Cholate	3.0	30	30.0
Oleic Acid, NF	1.0	10	10.0
Sucralose, 25%	0.8	8	8.0
Strawberry Flavor	0.4	4	4.0
Capric Acid	0.5	5	5.0
Lauric Acid	0.5	5	5.0
Citric Acid Anhydrous, USP	6.72	67.2	67.2
Trolamine, NF	5.22	52.2	52.2
Tromethamine, USP	4.24	42.4	42.4
Sodium Hydroxide, NF	1.88	18.8	18.8
Sodium Hydroxide, 5 N	QS	QS	QS
Hydrochloric Acid, 5N	QS	QS	QS
Sterile Water for Irrigation, USP	QS	QS	QS
Total	**100%**	**1.0 mL**	**1077.4 g**

1. Weight adjusted for protein content.

Preparation of Premix for HIM2 Oral Liquid

1. Add 94.3% of the tromethamine and the trolamine, citric acid and sodium hydroxide (NF) to 350 g sterile water for irrigation and stir until completely dissolved.
2. Moderately heat and maintain the temperature through steps 3 & 4, below.
3. Add the sodium cholate to step 2 and stir until dissolved.
4. Add the oleic acid, capric acid, lauric acid, sucralose solution and strawberry flavor to step 3 and stir until dissolved.
5. Adjust the temperature to approximately room temperature.

6. Adjust the pH of step 5, if necessary, to 7.8± 0.1 using 5N sodium hydroxide or 5N hydrochloric acid.
7. QS to the pre-mix batch weight with sterile water for irrigation.
8. Filter the step 7 product.

Preparation of HIM2 Oral Liquid, 6 mg/mL

1. Dispense the required quantity of the Premix for HIM2 Oral Liquid and continue stirring while performing steps 2 through 4 below.
2. Add the remaining tromethamine to 140 g of the sterile water for irrigation and stir until dissolved.
3. Adjust the pH of step 2, if necessary, to 7.7±0.2 using 5N sodium hydroxide or 5N hydrochloric acid.
4. Filter the step 3 liquid.
5. Add all of the HIM2 to step 4.and stir until completely dissolved.
6. Add all of step 5 to step 1 and stir.
7. Adjust the temperature to approximately room temperature, if necessary
8. Adjust the pH of step 7, if necessary, to 7.6-7.9 using 5N sodium hydroxide or 5N hydrochloric acid.

Example 12

HIM2 Oral Tablet Process

A general procedure to manufacture an oral tablet formulation of the present invention is shown below: The process involves making a lyophilized powder, adding tableting excipients and compressing.

Quantitative Composition of HIM2 Oral Tablets, 10 mg

Excipient Lyo Portion	Quantity per Batch (g)
HIM2	2.50[1]
Sodium Cholate	30.0
Oleic Acid, NF	10.0
Sucralose, 25%	8.0
Strawberry Flavor	4.0
Capric Acid	5.0
Lauric Acid	5.0
Citric Acid Anhydrous, USP	67.2
Trolamine, NF	52.2
Tromethamine, USP	42.4
Sodium Hydroxide, NF	18.8
Sodium Hydroxide, 5 N	QS
Hydrochloric Acid, 5N	QS
Sterile Water for Irrigation, USP	QS
Total	**1077.4 g**

Tablet Portion	
Lyo Portion	127.6
Citric Acid	29,7
Sodium Citrate dihydrate	84.2
Tris Base (tris(hydroxymethyl)aminomethane)	106.7
Microcrystilline Cellulose	24.8
Explotab	9.4
Total	**382.3**

1. Weight adjusted for protein content.

Procedure:

1. Dispense the required ingredients with the exception of the Sterile Water for Irrigation, USP,
2. Dispense 1500 g of Sterile Water for Irrigation, USP, and add to the processing vessel above.
3. Add the following ingredients to step 2 and mix until dissolved completely:
   a. all of the Sodium Cholate
   b. all of the Dibasic Sodium Phosphate Heptahydrate, USP
4. Add the following ingredients to step 3 and mix vigorously.

a. All of the Capric Acid
b. All of the Lauric Acid
c. All of the Sodium Hydroxide, NF (Note: Heat will be generated by the addition of Sodium Hydroxide, NF.)

5. Adjust the step 4 solution to a temperature between 45°C and 50°C, and mix until a clear solution results.
6. Cool to room temperature and, if necessary, adjust the step 5 pH to 7.2 – 7.8 using Sodium Hydroxide, 1N, or Hydrochloric Acid, 1N.
7. Add all of the HIM2 PEG 7 to step 6 and mix vigorously until a clear solution results.
8. Determine the amount of additional Sterile Water for Irrigation, USP, to add (if necessary)
9. Lyophilize this solution until a white amorphous powder results.
10. Blend the lyo portion with the tableting excipients.
11. Compress on a tablet press to achieve desired size, shape and hardness.

Example 13

Calcitonin-Oligomer Conjugate Oral Liquid Process

A general procedure to manufacture an oral liquid pharmaceutical composition of the present invention is shown below: The process involves making a premix without the drug, filtering the premix, then adding the premix and drug solution together.

Quantitative composition of CT-025 Oral Liquid, 25 ug/mL

Ingredient	Concentration (%w/w)	Concentration (mg/mL)
Modified calcitionin as protein	0.00242	0.025
Capric acid	1.72	17.8
Lauric acid	2.00	20.7
Sodium hydroxide, NF	0.77	7.96
Sodium cholate	4.30	44.4
Sucralose solution, 25% liquid concentrate	2.00	20.7

Strawberry flavor No. 45056	0.500	5.17
Sodium chloride, USP	0.200	2.07
Sodium phosphate, monobasic, monohydrate, USP	0.460	4.76
Sodium phosphate, dibasic, heptahydrate, USP	4.92	50.9
Sterile water for irrigation, USP	qs to 100%	qs to 1 mL

* CT-025 amount (25 µg/mL) is adjusted based on salmon calcitonin equivalents

CT-025 Oral Liquid Process

Method of Manufacture of Concentrated Base Solution

1. Place sterile water for irrigation, USP (80% of batch amount) in a stainless steel container.
2. Add the buffer components, sodium phosphate monobasic and dibasic, to the water and mix to dissolve.
3. Add the sodium cholate to the solution in step 2 and mix to dissolve.
4. Add the sodium hydroxide pellets, NF to the solution in step 3 and mix to dissolve. Adjust the temperature to 55 –60°C before proceeding to next step.
5. Add the fatty acids, capric acid and lauric acid, to the solution in step 4 and maintain the temperature at 55 –60°C until ingredients are completely dissolved. Then adjust the temperature of the liquid to 23 –27°C.
6. Add the taste masking ingredients: sucralose, strawberry flavor, and sodium chloride, to the solution in step 6. Mix to dissolve.
7. QS with sterile water for irrigation.
8. Filter solution through a 5-micron filter to remove any particulate matter.
9. Keep the base solution in a sealed container until ready for use to make the final oral liquid formulation.

Manufacture of CT-025 Oral liquid

10. Place portion of base solution in a stainless steel container. Start mixing and

continue mixing throughout processing.

11. Dispense sterile water for irrigation in a separate container. Add the amount of CT-025 required based upon protein content value to the water and mix to dissolve.

12. Add CT-025 solution in step 11 to base solution in step 10. Rinse container with sterile water and add rinse to solution provided in step 12.

13. QS with sterile water for irrigation.

Example 14

Calcitonin-Oligomer Conjugate Oral Tablet Process

A general procedure to manufacture an oral tablet formulation of the present invention is shown below: The process involves making a lyophilized powder, adding tableting excipients and compressing.

Quantitative composition of CT-025 Oral Tablets, 53 to 320 ug/tab

Ingredient	% w/w
Modified calcitonin as Protein	0.006-0.036
Milled Powder Blend*	50.3
Mannitol	34.6
Croscarmellose sodium	10.1
Sorbitol	5.03
Sodium Stearyl Fumarate	0.50
Sorbitol	

*composed of sodium cholate, sodium caprate and sodium laurate

CT-025 Oral Tablets Process.

1. Weigh out and dispense mannitol, sorbitol, and croscarmellose sodium in sufficient quantities to produce blend for all four of the development batches.
2. Pass the mannitol, sorbitol, and croscarmellose sodium through a #10 screen and layer the excipients in both arms of a 32 quart V-blender, alternating part of the Jet Milled Penetration Enhancer Blend with another excipient.
3. Blend for approximately 10 minutes.
4. Discharge the blend into a tared container lined with a LDPE bag and label as "Bulk Blend of Excipients".

5. Divide the blend into four equal parts by weighing out the blend into a tared container lined with a LDPE bag.

Wet granulation (Prepared as two sublots A and B):

Preparation of each sublot

6. Weigh 626.85 g of the "Bulk Blend of Excipients" and transfer into the key granulator.
7. Weigh half the required amount of CT-025* for the first sublot and transfer into a clean glass container.

   * Note: The CT-025 is removed from the freezer and left "as is" at ambient conditions for approximately 1 hour. The CT-025 is equilibrated by removing the lid of the bottle for at least 2 hours prior to weighing.
8. Pipette out 45 mL of 1% acetic acid solution into the glass container. Dissolve the CT-025 by swirling gently from side to side.
9. Start the granulation and add the CT-025 solution as the granulating fluid using a 60-cc syringe with a needle.
10. Add more 1% acetic acid solution as needed using a 10-cc syringe with needle.
11. Transfer the wet granules from the granulator into a LDPE bag.

Wet granulation (Prepared as two sublots A and B), continued:

Preparation of each sublot

12. Layer a tray with aluminum foil and spread the wet granules evenly on the foil. Put into the oven previously set at 37°C.
13. Repeat steps 6-12 for second sublot.

Drying:

14. Dry granules in an oven at 37°C for approximately 16 – 24 hours. Determine Loss On Drying. Each sublot is dried separately. Target moisture content is <3% upon removal from the oven.

Milling:

15. The dry granules of each sublot are passed through a #10 or #12 sieve.
16. The coarse granules retained are passed through a Quadro Comil equipped with a 0.125" screen.

17. Mix the sublots after the milling.
18. Weigh the total amount of granules collected.

Blending of Lubricant:

19. Load a 8 quart V-blender with granules from sublot A and B. Blend for approximately 5 minutes.
20. Weigh the required amount of sodium stearyl fumarate.
21. Remove one scoop of the granulation from each arm of the blender into a LDPE bag.
22. Add the sodium stearyl fumarate to the granules in the blender divided into both arms. Replace the granulation scooped out covering the sodium stearyl fumarate. Blend for approximately 2 minutes.
23. Discharge the final blend into a tared container lined with a LDPE bag. Sample final blend from various locations in the bag. Double bag the final blend for storage and label according to the sample label.

Tableting:

24. Use a single station press with caplet tooling to produce target weight per tablet of 900 mg (± 5% , 855-945 mg).

Example 15

Liquid oral pharmaceutical compositons formulated in accordance with Example 11 above were administered to healthy human volunteers in a 4-way crossover study both pre-prandial and post-prandial. The insulin conjugate HIM2 was provided at concentrations of 0.125. 0.25 and 0.5 mg/ml and these were compared to baseline values where no dosing occured. 20 mL oral doses were provided followed by 70 mL of water.

For the pre-prandial phase (fasted), subjects were fasted overnight and a single dose was administered in the morning. If blood glucose levels fell below 50 mg/dL, the subjects were rescued with a dextrose infusion. The effect of the conjugate HIM2 on blood glucose levels is illustrated in Figure 1. HIM2 plasma levels (expressed in insulin equivalents) are shown in Figure 2. The glucose/dose response and HIM2/dose response is shown in Figure 3.

For the post-prandial phase (fed), subjects received a single dose followed 15 minutes later by a meal. If blood glucose levels fell below 50 mg/dL, the subjects were rescued with a

dextrose infusion. The effect of the conjugate HIM2 on blood glucose levels is illustrated in Figure 4. HIM2 plasma levels (expressed in insulin equivalents) are shown in Figure 5. The glucose/dose response and HIM2/dose response is shown in Figure 6.

Example 16

Liquid oral pharmaceutical compositons formulated in accordance with Example 11 above were administered to male CF-1 mice (~20-25 g). The animals were fasted overnight and deprived of food during the experiment. Water was provided ad libitum. The mice were maintained in cages with 5 animals per cage and kept in a room with a 12:12 L:D cycle (6:00 a.m.-6:00 p.m.). The mice were tested in groups of 5 animals per dose. Each group of mice (N=5) received either insulin conjugate-075, insulin conjugate-076, insulin-conjugate-084, insulin conjugate-106 or HIM2 orally at 1.25 and 2.5 mg/kg.

The insulin conjugates and HIM2 were provided at concentrations of 0.125 and 0.25 mg/ml. The dosing volume was 10.0 mL/kg. Total doses (each compound) animals received were 1.25 and 2.5 mg/kg. Oral doses were administered using a gavaging needle (Popper gavage needle for mice #20; 5 cm from hub to bevel). Effect of the various conjugates on blood glucose level is illustrated in Figures 7, 8, and 9.

Example 17

Adult, male beagles dogs having a body weight of 8-15 kg were fasted overnight. The dogs were distributed equally by body weight among treatment groups with N=4/group. Tap water was provided *ad libitum*. The dogs were maintained in their assigned groups per protocol. The dogs were tested in groups of 4 animals per dose. The route of administration was bolus oral administration. Each group of dogs (n=4) received CT-025 at 25 μg/kg of liquid or ~10-23 μg/kg of tablet formulations in groups of 4 animals per dose. The dosing volume was 0.2 mL/kg. Liquids were administered by applying to the back of the throat using an appropriate-sized gavaging syringe. One or two tablets were administered per dog. The CT-025 was provided at concentrations of 125 ug/mL of liquid formulation or 125 ug per tablet weight. The dogs were re-fed approximately one hour after the final blood sampling.

Figure 10 illustrates the effect of CT-025 conjugate administered as described above using the following liquid formulation, which was prepared using a procedure similar to that described above in Example 13.

Ingredient	Percent
CT-025	125 µg/ml
Phosphate buffer	88.84
Sodium caprate	1.94
Sodium laurate	2.22
Sodium chloride	0.2
Sodium cholate	4.3
Sucralose	2
Strawberry flavor	0.5

Figure 11 illustrates the effect of CT-025 conjugate administered as described above using the following liquid formulation, which was prepared using a procedure similar to that described above in Example 13.

Ingredient	Percent
CT-025	125 µg/ml
Phosphate buffer	88.51
Capric acid	1.72
Lauric acid	2
Sodium chloride	0.2
Sodium cholate	4.3
Sodium hydroxide pellets	0.77
Sucralose	2
Strawberry flavor	0.5

Figure 12 illustrates the effect of CT-025 conjugate administered as described above using a formulation similar to the one described above in Example 14.

Example 18

Oral Dosing of C-Peptide Conjugate & Its Blood Levels in Mice

Mice (fasted over-night) were orally administered C-peptide conjugate (1.92 mg/kg bw) in a liquid formulation (200 µg/mL) similar to that described above in Example 11. The C-peptide conjugate was human C-peptide, having glutamate at its N-terminus and lysine at its C-terminus, with an oligomer having the structure $CH_3O(C_2H_4O)C(O)$- attached by an amide bond to the lysine of the human C-peptide. At each time intervals of 15, 30, 45, & 60 minutes, mice were anesthetized with ether and blood collected from the vena cava with

heparinized syringes. Plasma was separated, stored at $-80\ ^0$C, and transferred for analysis of C-peptide conjugate in plasma. The plasma effects of the orally administered C-peptide conjugate are shown in Figure 13.

The present invention has been described herein with reference to its preferred embodiments. These embodiments do not serve to limit the invention, but are set forth for illustrative purposes. The scope of the invention is defined by the claims that follow.

That Which is Claimed is:

1. A pharmaceutical composition comprising:

an drug-oligomer conjugate comprising a drug covalently coupled to an oligomeric moiety;

between 0.1 and 15% (w/v) of a fatty acid component;

between 0.1 and 15% (w/v) of a bile salt component;

wherein the fatty acid component and the bile salt component are present in a weight-to-weight ratio of between 1:5 and 5:1.

2. The pharmaceutical composition according to claim 1, wherein the fatty acid component and the bile salt component are present in a weight ratio of between 1:2 and 2:1.

3. The pharmaceutical composition according to claim 1, wherein the bile salt component comprises a pharmaceutically acceptable salt of cholic acid.

4. The pharmaceutical composition according to claim 1, wherein the bile salt component is sodium cholate.

5. The pharmaceutical composition according to any one of claims 1 to4, wherein the fatty acid component comprises a medium-chain fatty acid component and a long-chain fatty acid component.

6. The pharmaceutical composition according to any one of claims 1 to 4, wherein the pH of the composition is between 6.2 and 9.0.

7. The pharmaceutical composition according to any one of claims 1 to 4, wherein the drug is a calcitonin polypeptide.

8. The pharmaceutical composition of according to claim 7, wherein the calcitonin polypeptide is salmon calcitonin.

9. The pharmaceutical composition according to claim 8, wherein the drug-oligomer moiety comprises salmon calcitonin coupled to two oligomeric moieties, wherein

one oligomeric moiety is coupled to the lysine at the 11 position of the salmon calcitonin, and wherein one oligomeric moiety is coupled to the lysine at the 18 position of the salmon calcitonin.

10. The pharmaceutical composition of Claim 9, wherein the oligomeric moiety coupled to the lysine at the 11 position comprises the structure $CH_3O(C_2H_4O)_7$-$(CH_2)_7$-C(O)- and wherein the oligomeric moiety coupled to the lysine at the 18 position of the salmon calcitonin comprises the structure $CH_3O(C_2H_4O)_7$-$(CH_2)_7$-C(O)-.

11. The pharmaceutical composition according to any one of claims 1 to 4, wherein the drug-oligomer conjugate is present as a substantially monodispersed mixture.

12. The pharmaceutical composition according to any one of claims 1 to 4, wherein the drug-oligomer conjugate is present as a monodispersed mixture.

13. The pharmaceutical composition according to any one of claims 1 to 4, further comprising a buffering component.

14. The pharmaceutical composition according to claim 13, wherein the buffering component comprises tris-base and trolamine.

15. The pharmaceutical composition according to any one of claims 1 to 4, wherein the pharmaceutical composition is a solid dosage pharmaceutical composition.

16. The pharmaceutical composition according to any one of claims 1 to 4, wherein the pharmaceutical composition is a liquid pharmaceutical composition.

17. The pharmaceutical composition according to claim 16, wherein the liquid pharmaceutical composition is suitable for oral administration.

18. The pharmaceutical composition according to any one of claims 1 to 4, wherein the fatty acid component is present in an amount sufficient to lower the precipitation point of the bile salt compared to a precipitation point of the bile salt if the fatty acid component were not present in the pharmaceutical composition, and wherein the bile salt

component is present in an amount sufficient to lower the solubility point of the fatty acid compared to a solubility point of the fatty acid if the bile salt were not present in the pharmaceutical composition.

19. The pharmaceutical composition according to any one of claims 1 to 4, wherein the fatty acid component is present in a first amount such that, at the precipitation point of the bile salt, the bile salt precipitates as first bile salt particles that, upon a return to a pH above the precipitation point of the bile salt, re-solubilize more quickly than second bile salt particles that would have precipitated if the fatty acid component were not present in the composition.

20. The pharmaceutical composition according to any one of claims 1 to 4, wherein the bile salt component is present in an amount such that the fatty acid is soluble at a pH of 8.2, and wherein the fatty acid component is present in an amount such that the bile salt remains in solution at a pH of 5.5.

21. The pharmaceutical composition according to any one of claims 1 to 4, wherein the oligomeric moiety comprises a hydrophilic moiety and a lipophilic moiety.

22. The pharmaceutical composition according to any one of claims 1 to 4, wherein the drug-oligomer conjugate comprises the structure of Formula I:

$$\text{Drug}—B—L_j—G_k—R—G'_m—R'—G''_n—T \qquad \text{(I)}$$

wherein:

B is a bonding moiety;

L is a linker moiety;

G, G' and G" are individually selected spacer moieties;

R is a lipophilic moiety and R' is a polyalkylene glycol moiety, or R' is the lipophilic moiety and R is the polyalkylene glycol moiety;

T is a terminating moiety; and

j, k, m and n are individually 0 or 1.

23. The pharmaceutical composition according to any one of claims 1 to 4, wherein the drug-oligomer conjugate comprises the structure of Formula II:

$$\text{Drug}—X(CH_2)_mY(C_2H_4O)_nR \qquad \text{(II)}$$

wherein:

X is an ester moiety, a thio-ester moiety, an ether moiety, a carbamate moiety, a thio-carbamate moiety, a carbonate moiety, a thio-carbonate moiety, an amide moiety, a urea moiety or a covalent bond;

Y is an ester moiety, a thio-ester moiety, an ether moiety, a carbamate moiety, a thio-carbamate moiety, a carbonate moiety, a thio-carbonate moiety, an amide moiety, a urea moiety or a covalent bond;

m is between 1 and 24;

n is between 1 and 50; and

R is an alkyl moiety, a sugar moiety, cholesterol, adamantane, an alcohol moiety, or a fatty acid moiety.

24. The pharmaceutical composition according to any one of claims 1 to 4, wherein the drug-oligomer conjugate comprises the structure of Formula III:

$$\text{Drug}—X(CH_2)_m(OC_2H_4)_nOR \qquad \text{(III)}$$

wherein:

X is an ester moiety, a thio-ester moiety, an ether moiety, a carbamate moiety, thio-carbamate moiety, a carbonate moiety, a thio-carbonate moiety, an amide moiety, a urea moiety, or a covalent bond;

m is between 1 and 24;

n is between 1 and 50; and

R is an alkyl moiety, a sugar moiety, cholesterol, adamantane, an alcohol moiety, or a fatty acid moiety.

25. The pharmaceutical composition according to any one of claims 1 to 4, wherein the drug-oligomer conjugate comprises the structure of Formula IV:

$$\text{Drug-}\underset{H}{N}—\overset{\overset{O}{\|}}{C}\text{-}(CH_2)_m(OC_2H_4)_nOR \qquad \text{(IV)}$$

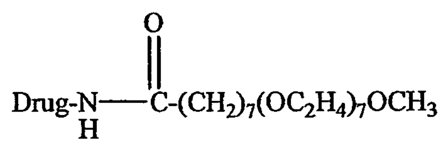
wherein:

m is between 1 and 24;

n is between 1 and 50; and

R is an alkyl moiety, a sugar moiety, cholesterol, adamantane, an alcohol moiety, or a fatty acid moiety.

26. The pharmaceutical composition according to any one of claims 1 to 4, wherein the drug-oligomer conjugate comprises the following structure:



27. A method of treating a bone disorder in a subject in need of such treatment, said method comprising administering to the subject a pharmaceutical composition comprising (a) a therapeutically effective amount of a calcitonin drug-oligomer conjugate that comprises a calcitonin drug covalently coupled to an oligomeric moiety; (b) between 0.1 and 15% (w/v) of a fatty acid component comprising a fatty acid; and (c) between 0.1 and 15% (w/v) of a bile salt component comprising a bile salt, wherein the fatty acid component and the bile salt component are present in a weight-to-weight ratio of between 1:5 and 5:1.

28. The method according to claim 28, wherein the fatty acid component and the bile salt component are present in a weight ratio of between 1:2 and 2:1.

29. The method according to claim 28, wherein the bile salt component comprises a pharmaceutically acceptable salt of cholic acid.

30. The method according to claim 28, wherein the bile salt component is sodium cholate.

31. The method according to any one of claims 28 to 31, wherein the fatty acid component comprises a medium-chain fatty acid component and a long-chain fatty acid component.

32. The method according to any one of claims 28 to 31, wherein the pH of the composition is between 6.2 and 9.0.

33. The method according to any one of claims 28 to 31, wherein the calcitonin drug is a calcitonin polypeptide.

34. The method according to claim 33, wherein the calcitonin polypeptide is salmon calcitonin.

35. The method according to claim 34, wherein the drug-oligomer moiety comprises salmon calcitonin coupled to two oligomeric moieties, wherein one oligomeric moiety is coupled to the lysine at the 11 position of the salmon calcitonin, and wherein one oligomeric moiety is coupled to the lysine at the 18 position of the salmon calcitonin.

36. The method of Claim 35, wherein the oligomeric moiety coupled to the lysine at the 11 position comprises the structure $CH_3O(C_2H_4O)_7$-$(CH_2)_7$-C(O)- and wherein the oligomeric moiety coupled to the lysine at the 18 position of the salmon calcitonin comprises the structure $CH_3O(C_2H_4O)_7$-$(CH_2)_7$-C(O)-.

37. The method according to any one of claims 28 to 31, wherein the calcitonin drug-oligomer conjugate is present as a substantially monodispersed mixture.

38. The method according to any one of claims 28 to 31, wherein the calcitonin drug-oligomer conjugate is present as a monodispersed mixture.

39. The method according to any one of claims 28 to 31, further comprising a buffering component.

40. The method according to any one of claims 28 to 31, wherein the buffering component comprises tris-base and trolamine.

41. The method according to any one of claims 28 to 31, wherein the pharmaceutical composition is a solid dosage pharmaceutical composition.

42. The method according to any one of claims 28 to 31, wherein the pharmaceutical composition is a liquid pharmaceutical composition.

43. The method according to any one of claims 28 to 31, wherein the method comprises orally administering the pharmaceutical composition.

44. The method according to any one of claims 28 to 31, wherein the oligomeric moiety comprises a hydrophilic moiety and a lipophilic moiety.

45. The method according to any one of claims 28 to 31, wherein the calcitonin drug-oligomer conjugate comprises the structure of Formula I:

$$\text{Calcitonin Drug}—B—L_j—G_k—R—G'_m—R'—G''_n—T \qquad (I)$$

wherein:

B is a bonding moiety;

L is a linker moiety;

G, G' and G" are individually selected spacer moieties;

R is a lipophilic moiety and R' is a polyalkylene glycol moiety, or R' is the lipophilic moiety and R is the polyalkylene glycol moiety;

T is a terminating moiety; and

j, k, m and n are individually 0 or 1.

46. The method according to any one of claims 28 to 31, wherein the calcitonin drug-oligomer conjugate comprises the structure of Formula II:

$$\text{Calcitonin Drug}——X(CH_2)_mY(C_2H_4O)_nR \qquad (II)$$

wherein:

X is an ester moiety, a thio-ester moiety, an ether moiety, a carbamate moiety, a thio-carbamate moiety, a carbonate moiety, a thio-carbonate moiety, an amide moiety, a urea moiety or a covalent bond;

Y is an ester moiety, a thio-ester moiety, an ether moiety, a carbamate moiety, a thio-carbamate moiety, a carbonate moiety, a thio-carbonate moiety, an amide moiety, a urea moiety or a covalent bond;

m is between 1 and 24;

n is between 1 and 50; and

R is an alkyl moiety, a sugar moiety, cholesterol, adamantane, an alcohol moiety, or a fatty acid moiety.

47. The method according to any one of claims 28 to 31, wherein the calcitonin drug-oligomer conjugate comprises the structure of Formula III:

$$\text{Calcitonin drug}—X(CH_2)_m(OC_2H_4)_nOR \qquad \text{(III)}$$

wherein:

X is an ester moiety, a thio-ester moiety, an ether moiety, a carbamate moiety, thio-carbamate moiety, a carbonate moiety, a thio-carbonate moiety, an amide moiety, a urea moiety, or a covalent bond;

m is between 1 and 24;

n is between 1 and 50; and

R is an alkyl moiety, a sugar moiety, cholesterol, adamantane, an alcohol moiety, or a fatty acid moiety.

48. The method according to any one of claims 28 to 31, wherein the calcitonin drug-oligomer conjugate comprises the structure of Formula IV:

$$\text{Calcitonin drug-}\underset{H}{N}—\overset{\overset{\displaystyle O}{\|}}{C}\text{-}(CH_2)_m(OC_2H_4)_nOR \qquad \text{(IV)}$$

wherein:

m is between 1 and 24;

n is between 1 and 50; and

R is an alkyl moiety, a sugar moiety, cholesterol, adamantane, an alcohol moiety, or a fatty acid moiety.

49. The method according to any one of claims 28 to 31, wherein the calcitonin drug-oligomer conjugate comprises the following structure:

$$\text{Calcitonin drug-}\underset{H}{N}—\overset{\overset{\displaystyle O}{\|}}{C}\text{-}(CH_2)_7(OC_2H_4)_7OCH_3$$

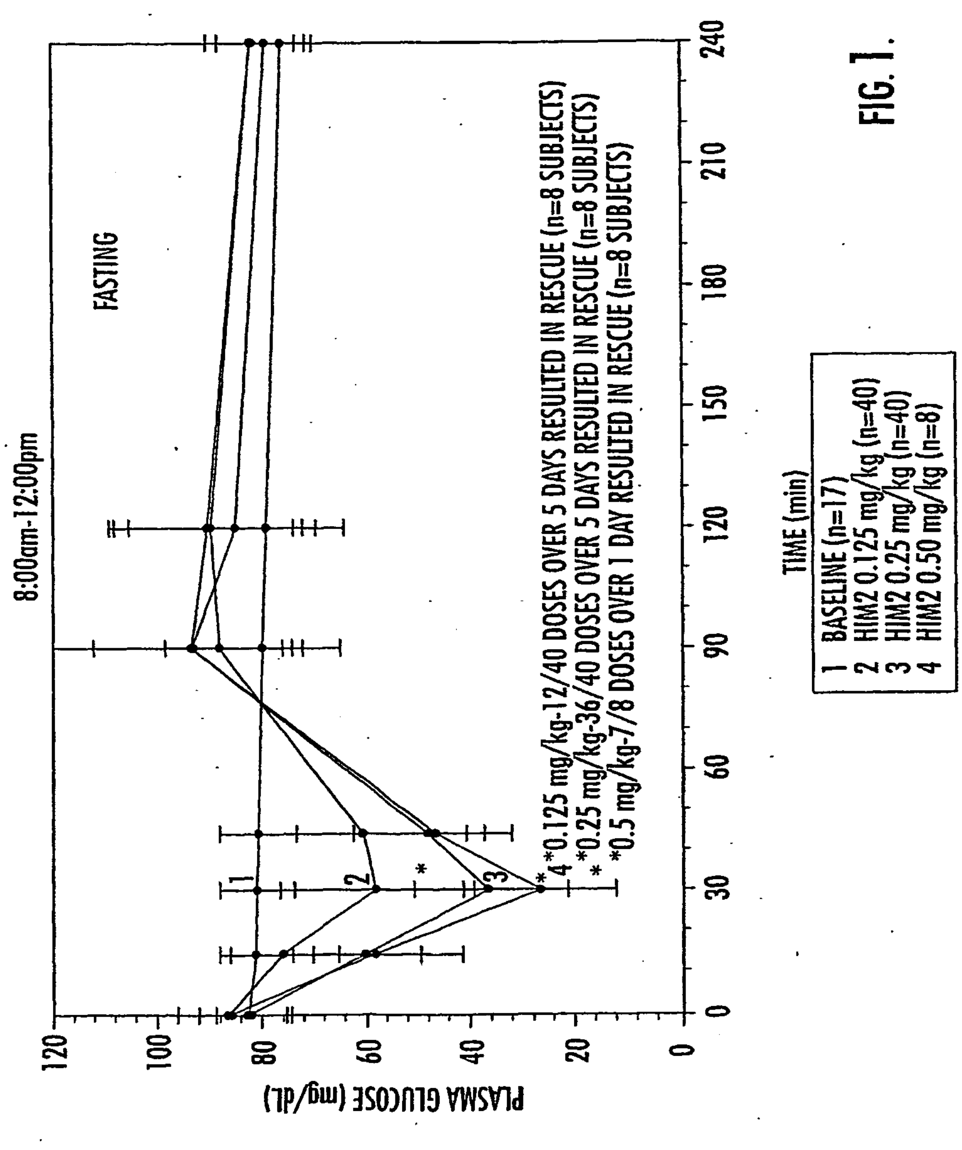

8:00am-12:00pm
FASTING
PLASMA GLUCOSE (mg/dL)
120
100
80
60
40
20
0
0
30
60
90
120
150
180
210
240
TIME (min)
4 *0.125 mg/kg-12/40 DOSES OVER 5 DAYS RESULTED IN RESCUE (n=8 SUBJECTS)
*0.25 mg/kg-36/40 DOSES OVER 5 DAYS RESULTED IN RESCUE (n=8 SUBJECTS)
*0.5 mg/kg-7/8 DOSES OVER 1 DAY RESULTED IN RESCUE (n=8 SUBJECTS)
1 BASELINE (n=17)
2 HIM2 0.125 mg/kg (n=40)
3 HIM2 0.25 mg/kg (n=40)
4 HIM2 0.50 mg/kg (n=8)

FIG. 1.

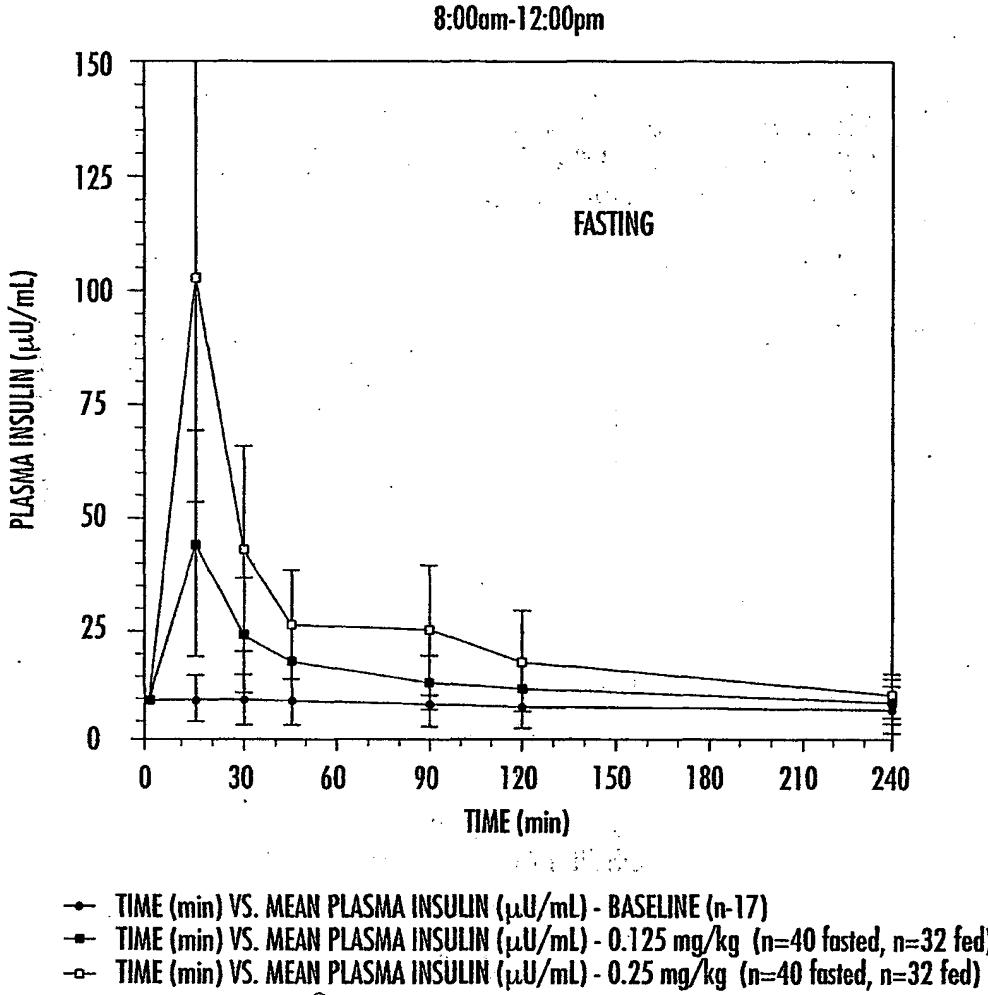

8:00am-12:00pm
150
125
100
75
50
25
0
PLASMA INSULIN (μU/mL)
FASTING
0 30 60 90 120 150 180 210 240
TIME (min)
TIME (min) VS. MEAN PLASMA INSULIN (μU/mL) - BASELINE (n-17)
TIME (min) VS. MEAN PLASMA INSULIN (μU/mL) - 0.125 mg/kg (n=40 fasted, n=32 fed)
TIME (min) VS. MEAN PLASMA INSULIN (μU/mL) - 0.25 mg/kg (n=40 fasted, n=32 fed)

FIG. 2.

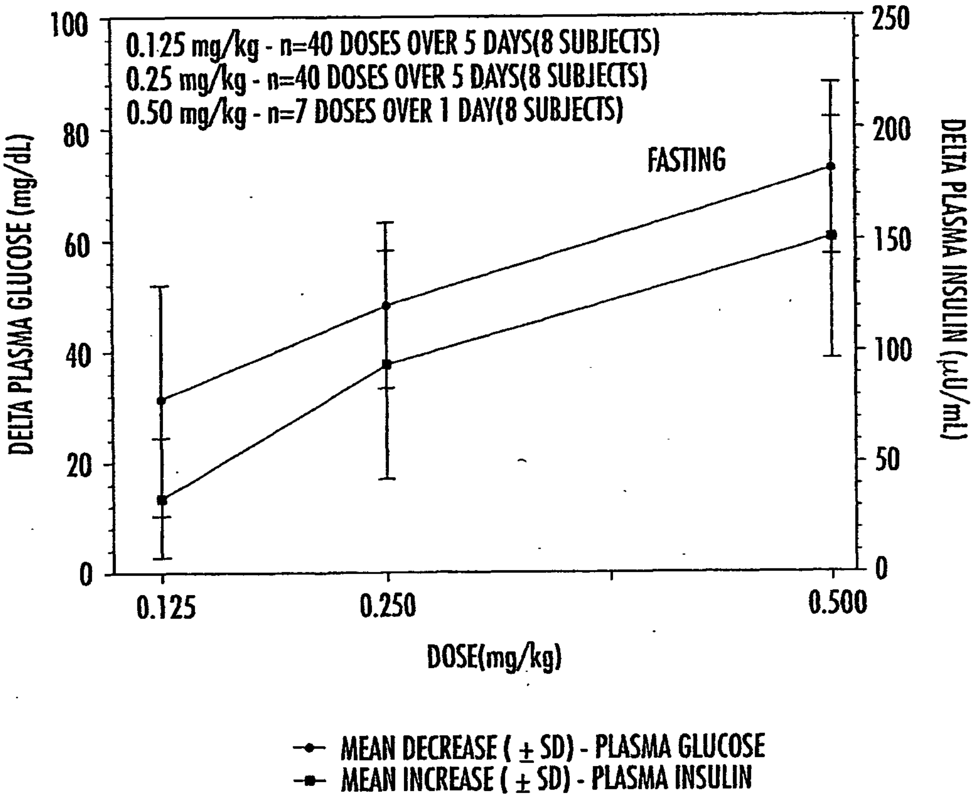

0.125 mg/kg - n=40 DOSES OVER 5 DAYS(8 SUBJECTS)
0.25 mg/kg - n=40 DOSES OVER 5 DAYS(8 SUBJECTS)
0.50 mg/kg - n=7 DOSES OVER 1 DAY(8 SUBJECTS)
FASTING
DELTA PLASMA GLUCOSE (mg/dL)
100
80
60
40
20
0
DELTA PLASMA INSULIN (μU/mL)
250
200
150
100
50
0
0.125
0.250
0.500
DOSE(mg/kg)
MEAN DECREASE (± SD) - PLASMA GLUCOSE
MEAN INCREASE (± SD) - PLASMA INSULIN

FIG. 3.

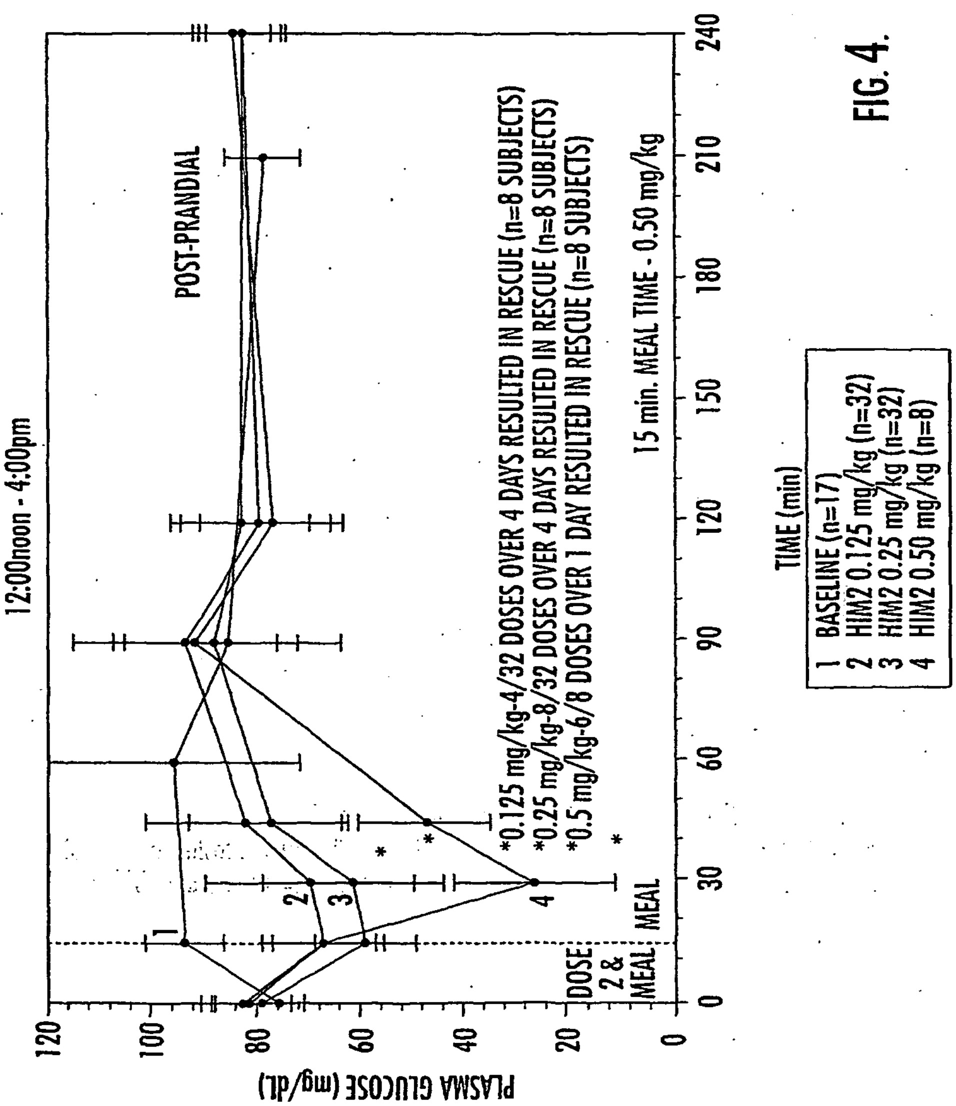
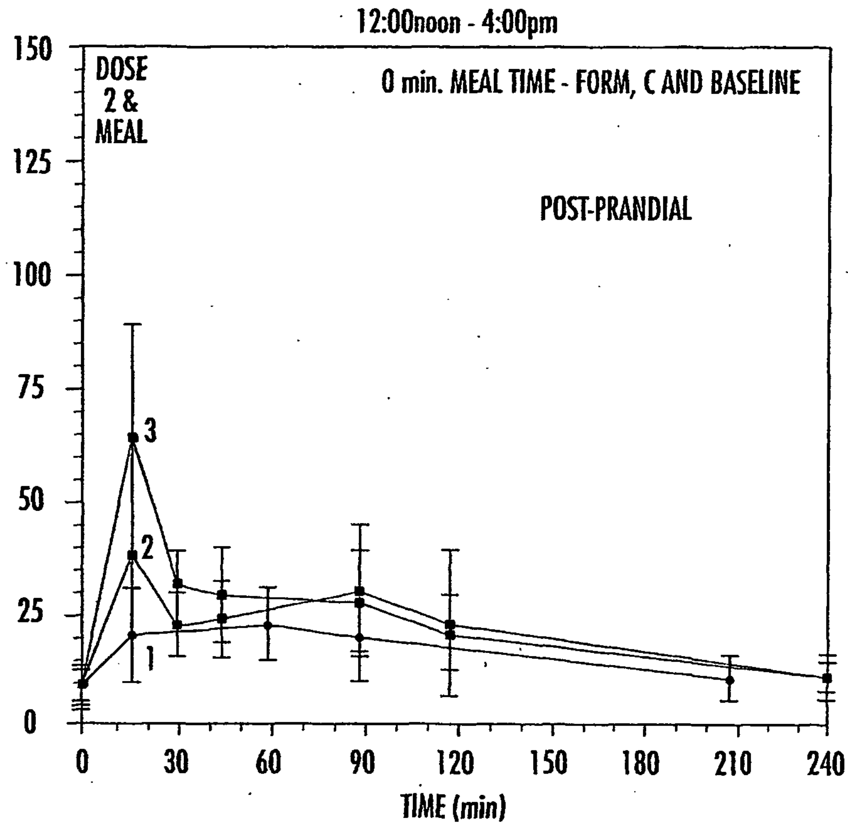

12:00noon - 4:00pm
POST-PRANDIAL
PLASMA GLUCOSE (mg/dL)
120
100
80
60
40
20
0
0
30
60
90
120
150
180
210
240
TIME (min)
DOSE 2 & MEAL
MEAL
*0.125 mg/kg-4/32 DOSES OVER 4 DAYS RESULTED IN RESCUE (n=8 SUBJECTS)
*0.25 mg/kg-8/32 DOSES OVER 4 DAYS RESULTED IN RESCUE (n=8 SUBJECTS)
*0.5 mg/kg-6/8 DOSES OVER 1 DAY RESULTED IN RESCUE (n=8 SUBJECTS)
15 min. MEAL TIME - 0.50 mg/kg
1 BASELINE (n=17)
2 HIM2 0.125 mg/kg (n=32)
3 HIM2 0.25 mg/kg (n=32)
4 HIM2 0.50 mg/kg (n=8)

FIG. 4.



1 TIME (min) VS. MEAN PLASMA INSULIN (μU/mL) - BASELINE (n-17)
2 TIME (min) VS. MEAN PLASMA INSULIN (μU/mL) - 0.125 mg/kg (n=40 fasted, n=32 fed)
3 TIME (min) VS. MEAN PLASMA INSULIN (μU/mL) - 0.25 mg/kg (n=40 fasted, n=32fed)

FIG. 5.

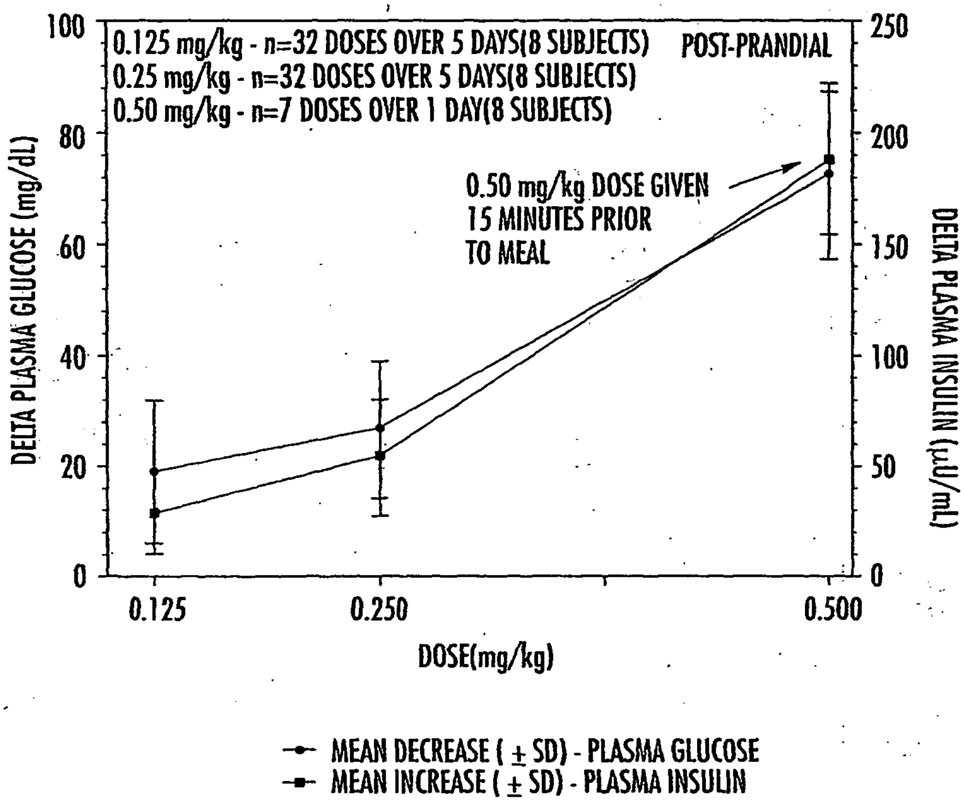

0.125 mg/kg - n=32 DOSES OVER 5 DAYS(8 SUBJECTS)
0.25 mg/kg - n=32 DOSES OVER 5 DAYS(8 SUBJECTS)
0.50 mg/kg - n=7 DOSES OVER 1 DAY(8 SUBJECTS)
POST-PRANDIAL
0.50 mg/kg DOSE GIVEN 15 MINUTES PRIOR TO MEAL
DELTA PLASMA GLUCOSE (mg/dL)
DELTA PLASMA INSULIN (μU/mL)
100
80
60
40
20
0
250
200
150
100
50
0
0.125
0.250
0.500
DOSE(mg/kg)
MEAN DECREASE (± SD) - PLASMA GLUCOSE
MEAN INCREASE (± SD) - PLASMA INSULIN

FIG. 6.

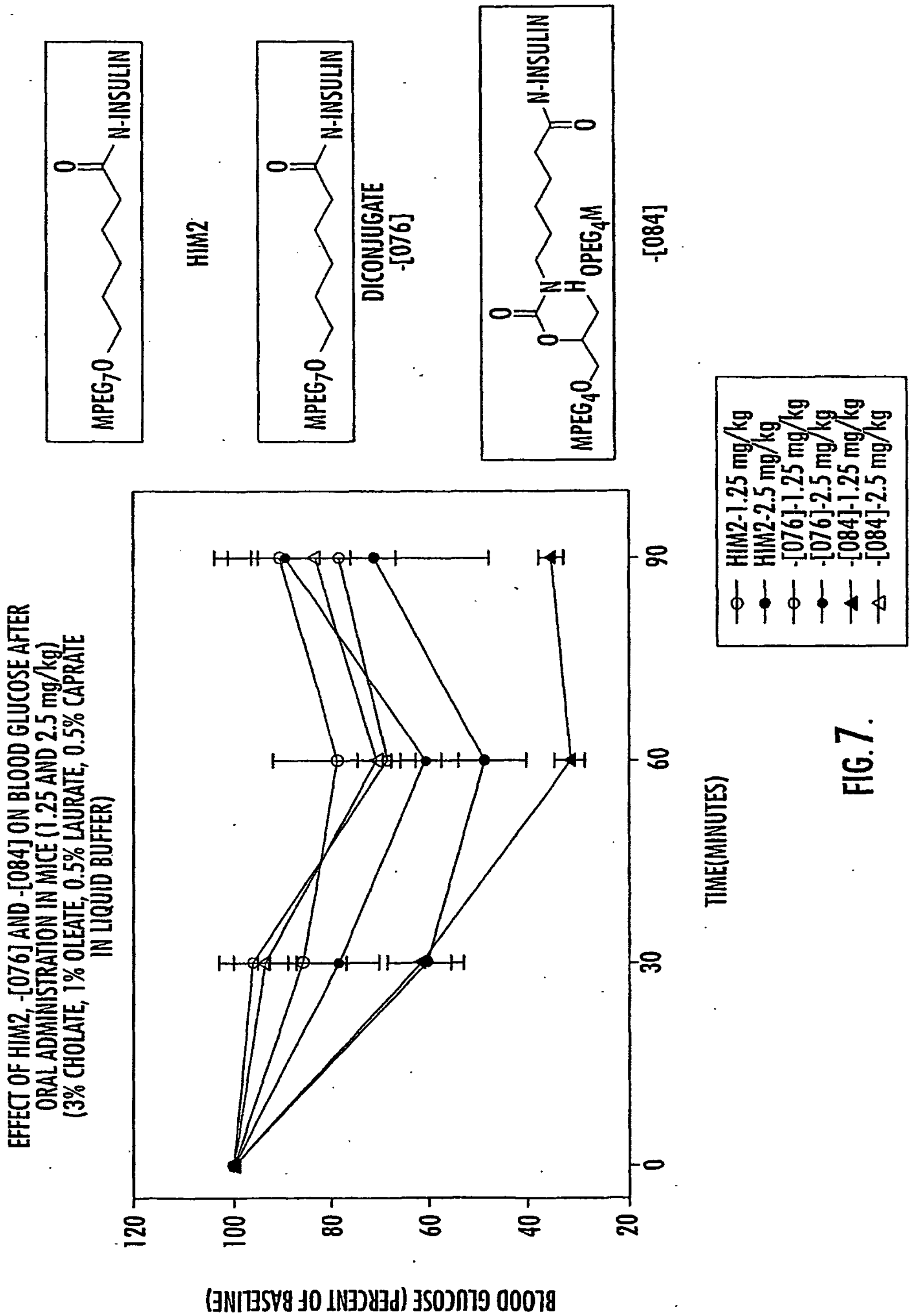

EFFECT OF HIM2, -[076] AND -[084] ON BLOOD GLUCOSE AFTER ORAL ADMINISTRATION IN MICE (1.25 AND 2.5 mg/kg) (3% CHOLATE, 1% OLEATE, 0.5% LAURATE, 0.5% CAPRATE IN LIQUID BUFFER)
BLOOD GLUCOSE (PERCENT OF BASELINE)
120
100
80
60
40
20
0
30
60
90
TIME(MINUTES)
HIM2-1.25 mg/kg
HIM2-2.5 mg/kg
-[076]-1.25 mg/kg
-[076]-2.5 mg/kg
-[084]-1.25 mg/kg
-[084]-2.5 mg/kg
MPEG7O
O
N-INSULIN
HIM2
MPEG7O
O
N-INSULIN
DICONJUGATE -[076]
MPEG4O
O
O
N
H
OPEG4M
O
N-INSULIN
-[084]

FIG. 7.

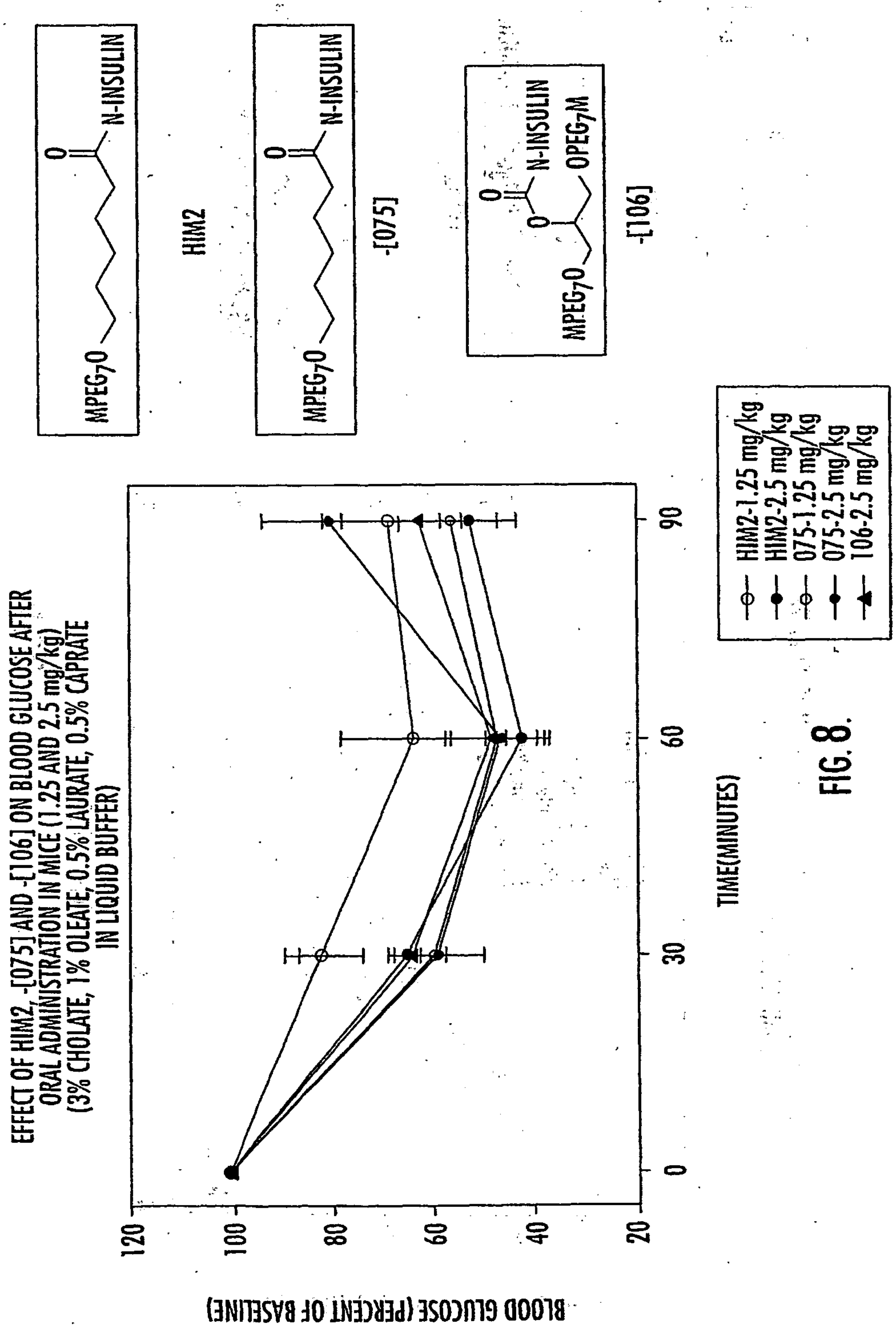
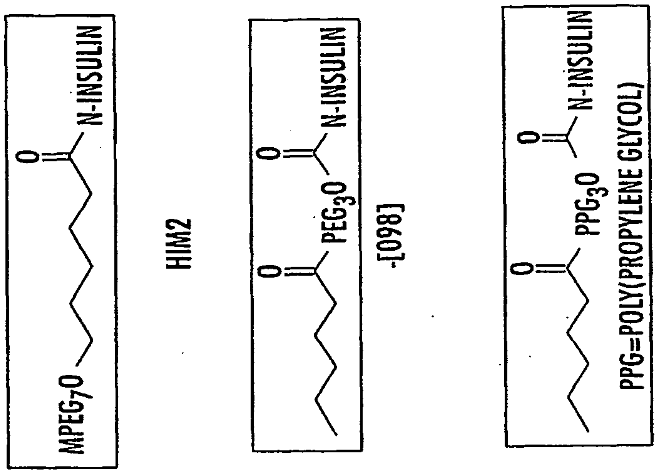

EFFECT OF HIM2, -[075] AND -[106] ON BLOOD GLUCOSE AFTER
ORAL ADMINISTRATION IN MICE (1.25 AND 2.5 mg/kg)
(3% CHOLATE, 1% OLEATE, 0.5% LAURATE, 0.5% CAPRATE
IN LIQUID BUFFER)
BLOOD GLUCOSE (PERCENT OF BASELINE)
120
100
80
60
40
20
0
30
60
90
TIME(MINUTES)
HIM2-1.25 mg/kg
HIM2-2.5 mg/kg
075-1.25 mg/kg
075-2.5 mg/kg
106-2.5 mg/kg
MPEG7O
N-INSULIN
O
HIM2
-[075]
OPEG7M
-[106]

FIG. 8.


MPEG$_7$O
O
N-INSULIN
HIM2
O
PEG$_3$O
O
N-INSULIN
-[098]
O
PPG$_3$O
O
N-INSULIN
PPG=POLY(PROPYLENE GLYCOL)
-[101]

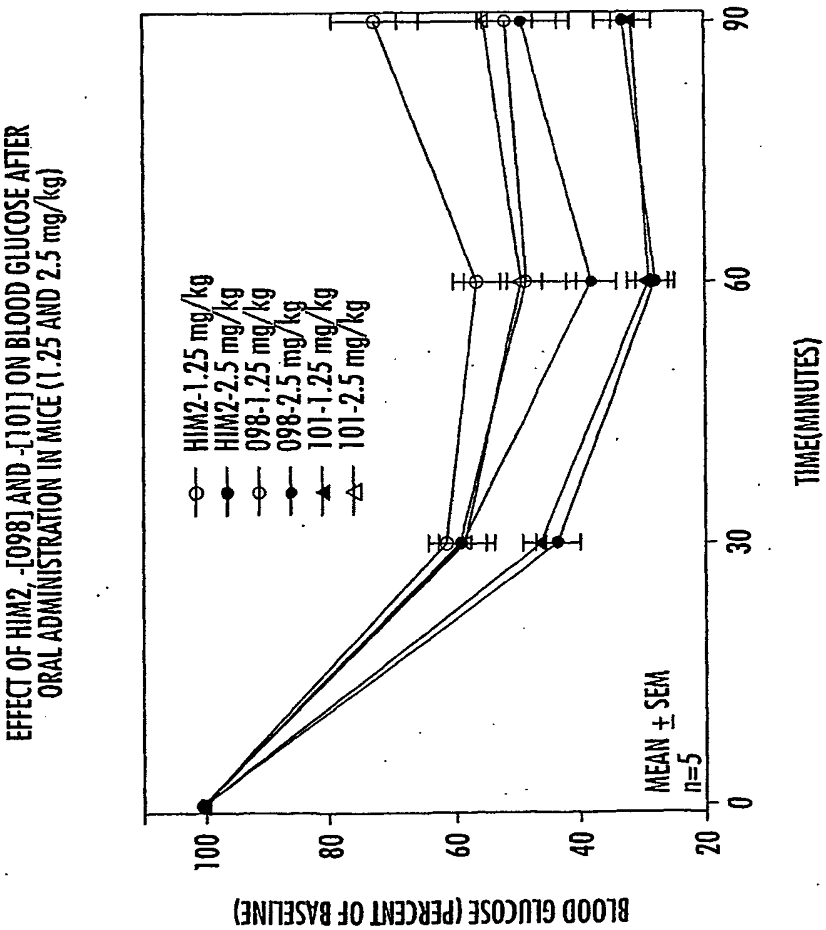

EFFECT OF HIM2, -[098] AND -[101] ON BLOOD GLUCOSE AFTER ORAL ADMINISTRATION IN MICE (1.25 AND 2.5 mg/kg)
HIM2-1.25 mg/kg
HIM2-2.5 mg/kg
098-1.25 mg/kg
098-2.5 mg/kg
101-1.25 mg/kg
101-2.5 mg/kg
MEAN ± SEM
n=5
100
80
60
40
20
BLOOD GLUCOSE (PERCENT OF BASELINE)
0
30
60
90
TIME(MINUTES)

FIG. 9.

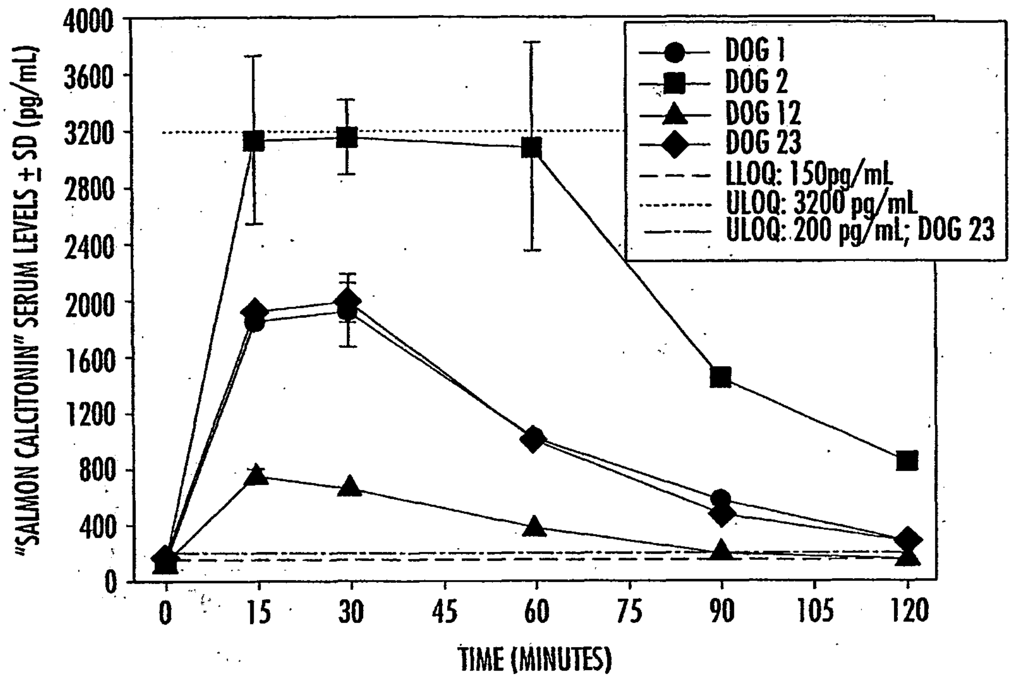

4000
3600
3200
2800
2400
2000
1600
1200
800
400
0
"SALMON CALCITONIN" SERUM LEVELS ± SD (pg/mL)
0
15
30
45
60
75
90
105
120
TIME (MINUTES)
DOG 1
DOG 2
DOG 12
DOG 23
LLOQ: 150pg/mL
ULOQ: 3200 pg/mL
ULOQ: 200 pg/mL; DOG 23

FIG. 10.

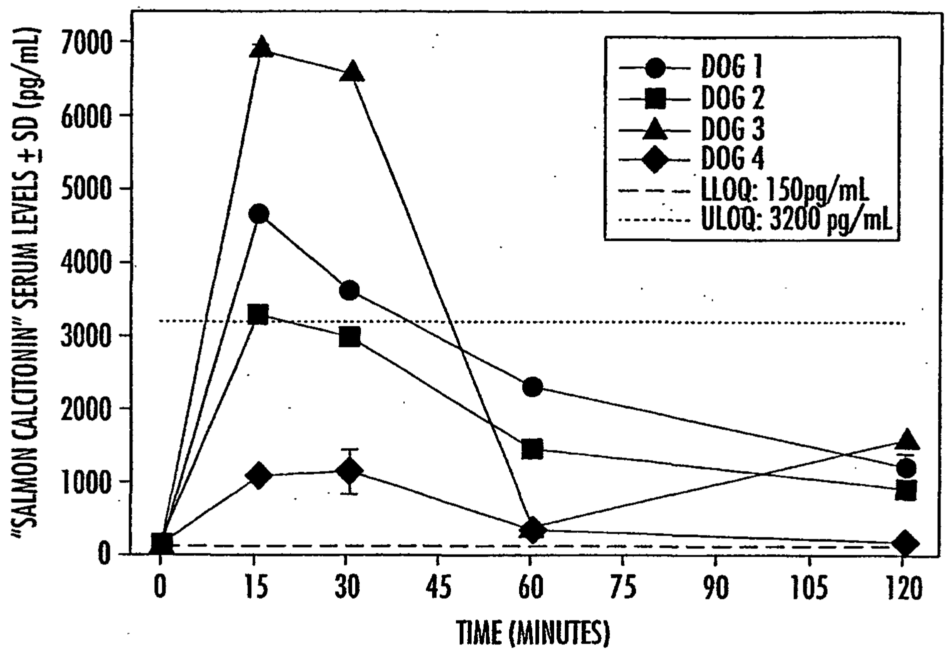

"SALMON CALCITONIN" SERUM LEVELS ± SD (pg/mL)
7000
6000
5000
4000
3000
2000
1000
0
0
15
30
45
60
75
90
105
120
TIME (MINUTES)
DOG 1
DOG 2
DOG 3
DOG 4
LLOQ: 150pg/mL
ULOQ: 3200 pg/mL

FIG. 11.

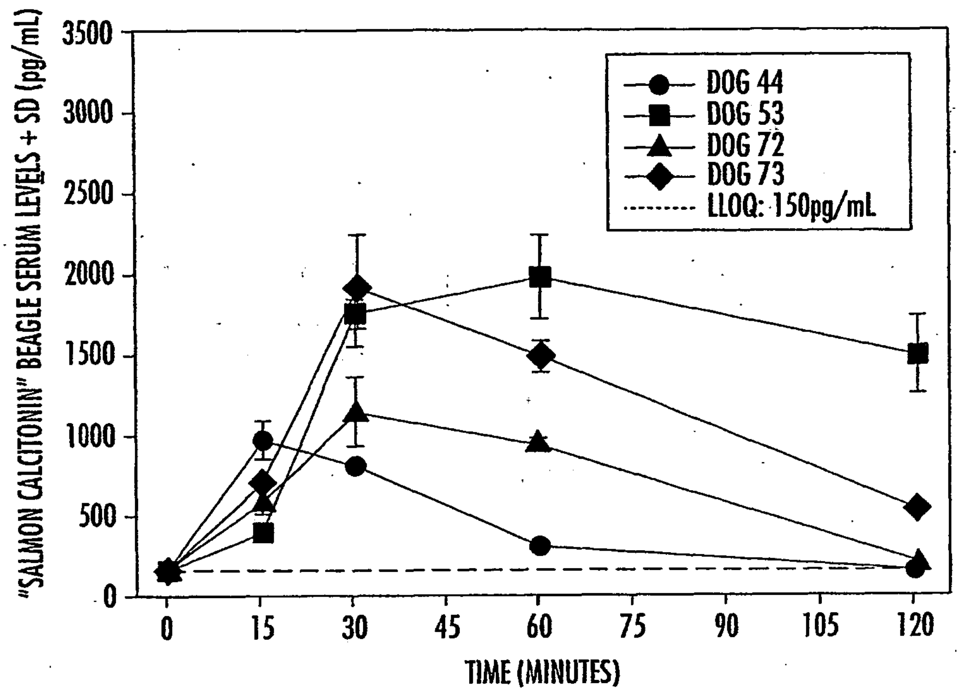

3500
3000
2500
2000
1500
1000
500
0
"SALMON CALCITONIN" BEAGLE SERUM LEVELS + SD (pg/mL)
0
15
30
45
60
75
90
105
120
TIME (MINUTES)
DOG 44
DOG 53
DOG 72
DOG 73
LLOQ: 150pg/mL

FIG. 12.

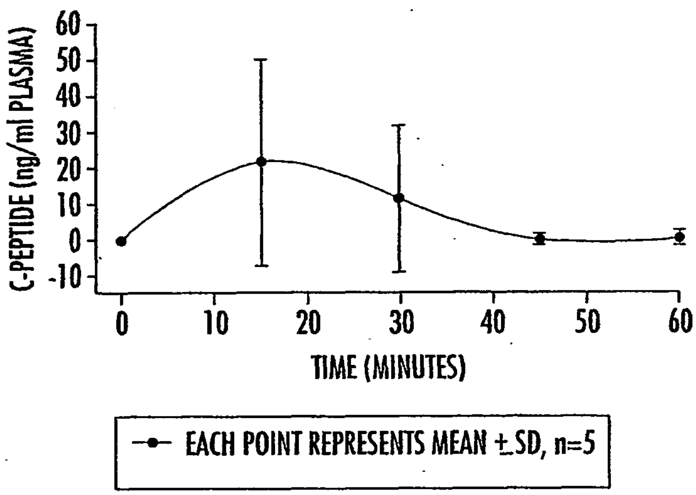

C-PEPTIDE (ng/ml PLASMA)
60
50
40
30
20
10
0
-10
0
10
20
30
40
50
60
TIME (MINUTES)
EACH POINT REPRESENTS MEAN ±SD, n=5

FIG. 13.